

Polyploidization study in wild wheat
by Arunrut Vanichanon

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Genetics
Montana State University
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Abstract:
Polyploidization is a key component of plant evolution. The number of independent origins of polyploid species has been traditionally underestimated. The objective of this work was to study multiple origins of tetraploid wild wheat. We screened 84 primer sets to identify genome-specific primer sets for the tetraploid wild wheat [Aegilops triuncialis (UC genome)] and its diploid progenitors [Ae. umbellulata (U genome) and Ae. caudata (C genome)]. Primer sets G12 and G43 were U genome-specific and D21 was a C genome-specific primer. Restriction fragment analysis and DNA sequence comparison were used to estimate the number of polyploidization events in the formation of Ae. triuncialis. G43 data revealed at least two independent formations of Ae. triuncialis. In the chloroplast hotspot region, results suggested that at least three polyploidization origins may have occurred independently. Despite evidence for multiple origins, less genetic variation was found in Ae. triuncialis than in its diploid progenitors. Ae. triuncialis appears to be a tetraploid with multiple origins with a minimal genome change after its formation.

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APPROVAL

Of a dissertation submitted by

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Approved for Department of Plant Sciences and Plant Pathology

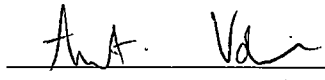
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ABSTRACT

Polyploidization is a key component of plant evolution. The number of independent origins of polyploid species has been traditionally underestimated. The objective of this work was to study multiple origins of tetraploid wild wheat. We screened 84 primer sets to identify genome-specific primer sets for the tetraploid wild wheat [*Aegilops triuncialis* (UC genome)] and its diploid progenitors [*Ae. umbellulata* (U genome) and *Ae. caudata* (C genome)]. Primer sets G12 and G43 were U genome-specific and D21 was a C genome-specific primer. Restriction fragment analysis and DNA sequence comparison were used to estimate the number of polyploidization events in the formation of *Ae. triuncialis*. G43 data revealed at least two independent formations of *Ae. triuncialis*. In the chloroplast hotspot region, results suggested that at least three polyploidization origins may have occurred independently. Despite evidence for multiple origins, less genetic variation was found in *Ae. triuncialis* than in its diploid progenitors. *Ae. triuncialis* appears to be a tetraploid with multiple origins with a minimal genome change after its formation.

The polymerase chain reaction (PCR) has become a standard procedure in plant genetics. One advantage of PCR is that sequence information for primer sets can be exchanged between labs, obviating the need for exchange and maintenance of biological materials. Repeatability of primer sets, whereby the same products are amplified in different labs using the same primer set, is important for successful exchange and utilization. We have developed several hundred sequence-tagged-site (STS) primer sets. The ability of the primer sets to generate reproducible amplifications in other laboratories has been variable. We wished to empirically determine the properties of the primer sets that most influenced repeatability. In our study, a total of 96 primer sets were tested with four genomic DNA samples on each of four thermocyclers. All major bands were repeatable across all four thermocyclers for approximately 50% of the primer sets. Characteristics most often associated with differences in repeatability included primer GC content and 3'-end stability of the primers. The propensity for primer-dimer formation was not a factor in repeatability. Our results provide empirical direction for the development of repeatable primer sets.

CHAPTER 1

INTRODUCTION

Wild Wheat

The genus *Aegilops* L. comprises more than 20 species including many diploid, allotetraploid and allohexaploid species (Kimber and Feldman, 1987; Slageren, 1994). Cultivated wheat, an allohexaploid species, belongs to the genus *Triticum* L. that is closely related to *Aegilops* L. Wheat is a broadly cultivated crop and an important staple food in the world (Vasil and Vasil, 1999). Studying the wild relatives of wheat may reveal important facts that can lead to a better understanding and improvement of cultivated wheat.

In the *Triticeae*, there was presumably a common ancestor which gave rise to all diploid *Aegilops* and *Triticum* species. Based on interspecific crosses, almost every species had a distinct genome because homeologous chromosomes of different species did not completely pair. Most natural interspecific hybrids were completely or almost completely sterile. All diploid *Aegilops* and *Triticum* species were genetically isolated from each other during evolutionary time (Kimber and Feldman, 1987).

Approximately 70 % of all angiosperms are of polyploid origin (Soltis and Soltis, 1999), including wheat. Since polyploidization is a major force in plant evolution, it is a crucial component for understanding plant evolution. There are several reviews of

polyploid evolution, including the mode and rate of formation of polyploids, ecological and evolutionary attributes and genetic consequences of polyploidy (Leitch and Bennet, 1997; Soltis and Soltis, 1999; Otto and Whitton, 2000; Wendel, 2000). Recent studies indicate that the number of polyploidization events involved in the origin of polyploid species has traditionally been underestimated (Soltis and Soltis, 2000; Ben-Ari, 1998). Several studies have indicated that the evolution of wheat underwent a bottleneck situation based on observed low levels of variation using RFLPs (Harcout and Gale, 1991; Kam-Morgan et al., 1989; Autrique et al., 1996) and isozyme (Asins and Carbonell, 1989; Nevo and Beiles, 1989). However, results of Talbert et al. (1998) revealed that wheat originated at least twice based on DNA sequence analysis. Multiple origins in other *Triticum* and *Aegilops* species still need to be examined.

This study focuses on *Ae. triuncialis* because of its remarkable adaptation. *Ae. triuncialis*, an allotetraploid species, is of particular interest because it has a wider geographical and ecological distribution than its diploid progenitors. *Ae. caudata*, a diploid parent, was described as a Mediterranean species which grows abundantly mainly in western Turkey. It is a typical lowland species. *Ae. umbellulata*, another diploid parent, is of Mediterranean and Western Asiatic origin and is uncommon throughout its range. On the other hand, *Ae. triuncialis ssp. triuncialis* has widespread distribution in the Mediterranean, Western Asiatic, southern Europe and central Asia. It is common throughout its range. Conversely, *Ae. triuncialis ssp. persica* is rare in west and central Asia.

Molecular genetic studies have partially elucidated the evolution of *Ae. triuncialis*. Murai and Tsunewaki (1986) used restriction fragment analysis to hypothesize that *Ae. triuncialis* resulted from reciprocal crosses of *Ae. umbellulata* and *Ae. caudata*. The genome relationships between *Aegilops* allopolyploids and their diploid ancestors was investigated using RFLP of chloroplast genome (Ogihara and Tsunewaki, 1988) and mitochondria genome (Ogihara et al., 1993). Waines and Barnhart (1992) proposed that the female genome should be listed first in the genome formulae. Therefore, the formulae for *Ae. triuncialis ssp. triuncialis* is proposed to be UUCC and CCUU for *Ae. triuncialis ssp. persica* (Slageren, 1994). In our study, *Aegilops triuncialis* was used as a plant model to illustrate multiple origins and genetic consequences of polyploidization using DNA sequence comparison

The Polymerase Chain Reaction (PCR)

Plant molecular biology currently relies on the polymerase chain reaction (PCR) to provide a nearly unlimited source of DNA molecules for further manipulation or subsequent experimental analyses. The powerful nature of PCR has led to significant insights into biological questions including those regarding plant evolution.

PCR is a relatively simple technique conceived in 1983 by Kary Mullis (Mullis and Faloona, 1987), involving a cycle process which leads to an exponential amplification of the target DNA. The goal of PCR is to replicate a particular DNA region, not the entire genome. This is achieved by using primers, which target a specific DNA

region. A primer is a short sequence of DNA, usually 18-25 base pairs (bp) long, that is complementary to one end of the target DNA. Both strands of the DNA target are copied by using two primers; one for the 5' end of the one strand and one for the 5' end of the complementary strand. The strands of the target DNA duplex are separated by heating and then cooled to allow primer annealing. Once the primers are annealed to the template, *Taq* DNA polymerase adds nucleotides complementary to the template. Extension is accomplished by increasing the temperature to the optimum for *Taq* DNA polymerase. Each heating and cooling cycle results in the doubling of the amount of template; therefore, after 20 cycles the yield of PCR product is approximately one million copies (2^{20}) of the single target DNA molecule. Annealing temperature, length of annealing and extension steps within each cycle, and cycle numbers vary for different target DNA regions and primers (Aert et al., 1998; Bruke, 1996).

PCR technology has several advantages for plant molecular biology research. Gene content and genetic map colinearity in grasses is well accepted (Bennetzen, 1999). Therefore, cross-species primers are widely used to accelerate research such as chloroplast primers among all land plants (Petit et al., 1998), and between barley and wheat (Erpelding et al., 1996). Furthermore, primers can be widely disseminated among researchers because they are easily synthesized from their sequences. One disadvantage of PCR is that *Taq* polymerase incorporates occasional errors at a rate of approximately 10^{-4} per base per doubling in newly synthesized DNA (Andre et al., 1997). In practical terms, this may not be a major problem because the errors are apparently not biased in

favor of any specific nucleotide. One way to cope with the potential error in DNA sequence comparison is to sequence both strands of molecule (Judd et al., 1999).

Molecular markers have become increasingly important in plant molecular biology in its relationship to plant breeding, plant systematics and plant evolution. Most molecular marker systems are presently based on PCR technology. The objective of our study is to discern the important characteristics of primers which influence reproducible results among laboratories.

Molecular Genetic Approaches in Plant Evolution

Molecular markers of various types have helped to increase understanding and elucidate new aspects of plant evolution. Isozymes have been widely used as genetic markers in plant diversity studies since 1959. Several advantages of isozymes are that they usually produce codominant markers, they are easy to assay in large populations and they monitor the genetic variation at specific gene loci. Some limitations are evident as well: the inability to detect water insoluble or cell structure-bound enzymes and the failure to distinguish isozyme bands with identical mobility that are in reality two different alleles (Muller-Starck, 1998). Additionally, only a small set of isozyme markers are available.

Molecular markers using restriction site analysis based on DNA sequence differences are common for studying variation. Cytoplasmic DNA is extracted and then digested with restriction enzymes followed by electrophoretic gel analysis. An advantage

of this technique is that analysis covers a large DNA region. However, a disadvantage is that different mutations may result in the same sized bands. Therefore, some results may lead to incorrect interpretation.

An adapted technique, restriction fragment length polymorphism (RFLP) analysis, requires digestion of nuclear and organellar DNA using restriction enzymes, blotting and hybridization with a radioactively labeled probe (Botstein et al., 1980). RFLP has been widely used for plant breeding, plant systematic and plant evolution for over two decades.

RFLP is a laborious process whereas other currently used molecular markers based on PCR are not. Furthermore, PCR fragments can be subjected to restriction site analysis. PCR technology has generated a variety of molecular markers to study plant evolution and diversity including PCR-single-strand conformational polymorphism (PCR-SSCP) (Wang et al., 1997), randomly amplified polymorphic DNAs (RAPDs) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Daly, 1998; Vos et al., 1995), sequence-tagged-site PCR (STS-PCR) (Talbert et al., 1996) and simple sequence repeats PCR (SSRs-PCR) (Pestsova et al., 2000). However, DNA sequencing is the ultimate method to detect genetic variation with the greatest detail. The current advances in automated sequencing continue to increase DNA sequence data. Thus, we selected DNA sequence analysis to study evolution questions in wild wheat.

CHAPTER 2

MULTIPLE ORIGINS AND GENETIC VARIABILITY OF ALLOPOLYPLOID *Aegilops triuncialis* USING NUCLEAR AND CHLOROPLAST MOLECULAR DATA

Introduction

Within the plant kingdom, polyploidization is a powerful force leading to speciation as well as an important source of genetic variation. In general, seventy percent of angiosperms have undergone polyploidization at least once (Soltis and Soltis, 1999). Polyploid species tend to be more widely distributed and found in more extreme habitats than their diploid ancestors (Soltis and Soltis, 2000). Understanding the number of polyploidization events which have occurred in the formation of a given species, and the consequences of such events, has been a major challenge. Although polyploidization has been studied for nearly a century, there are still many unknowns. The traditional point of view is that polyploidization events were rare because there is less variability within polyploid species than the diploid relatives such as wheat (Talbert et al., 1998). Putative examples of monophyletic origins include peanut *Arachis hypogaea* (Kochert et al., 1996) and salt marsh grass *Spartina anglica* (Raybould et al., 1991). However, more recently recurrent formation of polyploids has been demonstrated. Over 30 examples of allotetraploid species have been shown to have multiple origins to date (Soltis and Soltis, 1999). *Draba norvegica* (Brassicaceae) formed at least 13 times in a small area of

Scandinavia (Brochmann and Elven, 1992). *Tragopogon miscellus* and *T. mirus* have formed as many as 21 and 9 times, respectively, in eastern Washington and western Idaho, in the past 60 to 70 years (Soltis et al., 1995). Hexaploid wheat formed at least twice from *Triticum tauschii* (D genome) as a diploid progenitor (Talbert et al., 1998). The genetic and evolutionary consequence of multiple polyploidization within species still needs investigation.

Allopolyploids receive their chromosome sets from different species, unlike autopolyploids that receive multiple sets of chromosomes from one species. The prevalence of allopolyploid formation and the degree of genetic separation between allopolyploids and their progenitors have important consequences for the accumulation of genetic variability within an allopolyploid. Wheat and its wild relatives provide opportunity for studying allopolyploidization. For example, wheat, as an allohexaploid, has three genomes: A, B and D genomes. *Tragopogon miscellus* is the best known example of an allotetraploid species that occurred recently by reciprocal crosses of the diploids, *T. dubius* and *T. pratensis*, according to molecular data (Soltis et al., 1995). Like *Tragopogon miscellus*, *Aegilops triuncialis* (UC genome) is an allotetraploid that resulted from reciprocal crosses of diploids, *Ae. umbellulata* (U genome) and *Ae. caudata* (C genome) (Wang et al., 1997).

Aegilops triuncialis is the most widespread *Aegilops* species in the world. This grass is distributed between altitudes of 300 m and 1000 m and has become a troublesome weed on U.S. rangelands (Watanabe and Kawahara, 1999). Additionally, *Ae. triuncialis* is of agronomic interest as the source of resistance for leaf rust (*Puccinia*

triticina), Karnal bunt (*Ustilago tritici*), powdery mildew (*Erysiphe graminis*) and cereal cyst nematode (*Heterodera avenae*) resistance in cultivated wheat (Singh et al, 2000). Its tremendously successful adaptation results from genetic attributes of polyploids such as high genetic variability and the evolution of new gene functions (Soltis and Soltis, 2000).

Morphological, cytological and electrophoretic approaches are used to determine multiple origins of allopolyploid species. Molecular approaches including restriction fragment analysis, comparative sequencing and various PCR-based techniques are now available to uncover recurrent origins of allopolyploid. It is important to carefully select DNA regions when using these approaches because various genomes and DNA regions are suitable for different taxonomic levels (Soltis et al., 1998). Within a plant cell, three different types of DNA are found: nuclear, chloroplast and mitochondria DNA. Most current molecular data have come from the chloroplast, the highly repetitive sequences of ribosomal RNA (rRNA) and low copy genes (Soltis etal., 1998). The most frequently chosen nuclear DNA studied has been rRNA including 5S, 18S-26S rRNA, and internal transcribed spacer (ITS). Based on rRNA, Soltis and Soltis (1991) suggested multiple origins of the allopolyploid, *Tragopogon mirus*.

Because the nuclear genome is inherited biparentally, genome specific primers are preferred. Genome specific primers lessen ambiguous results by demonstrating direct inheritance of an allele from a specific ancestral genome. For example, using the D genome specific primer A1 sequence comparison between wheat and the D genome diploid progenitor indicated that hexaploid wheat was formed at least twice (Talbert et al., 1998).

Badaeva et al. (1996a) chose 5S and 18S-5.8S-26S(18S-26S) rRNA gene families to study the relationship of the U, C and D genomes among *Aegilops* diploid species. All three genomes have the same 5S rRNA banding pattern. The D genome had a unique pattern in 18S-5.8S-26S(18S-26S) rRNA. However, U and C genomes showed identical patterns. Highly repetitive nuclear DNA clones from rye and the D genome were also selected. The results indicated that U and C genomes were closely related; however, the D genome was more closely related to the M and M^h genomes than other *Aegilops* diploid species (Badaeva et al., 1996b). Another study used 46 probes of nuclear repetitive nucleotide sequences to resolve the relationship among *Aegilops* diploid species. The results showed that the U and C genomes were closely related and in the same clade (Dvorak and Zhang, 1992).

Because of the size and slow mutation rate of chloroplast genome, it has several advantages for taxonomic and evolutionary study. The genome is quite small, approximately 120-200 kilobases (kb). In Chinese Spring wheat, the chloroplast genome is 134,540 base pairs (bp) long (Ogihara and Tsunewaki, 2000). Chloroplast DNA is usually maternally inherited in plants. Most chloroplast genes are a single copy (Palmer et al., 1985). The genome is relatively conserved during its evolution throughout plant species. The chloroplast genome evolves four to five times slower than the nuclear genome and three times faster than mitochondria (Page and Holmes, 1998). Because of the slow mutation rate of plant mitochondria DNA, its genome was not selected in this study. Because different regions of DNA evolved at different rates, some parts of the chloroplast DNA might be appropriate to resolve relationships at different taxonomic

levels (Soltis and Soltis, 1998). For instance, some noncoding regions and microsatellites may be applicable at an intraspecific level due to their greater variation (Karp et al., 1998; Gielly and Taberlet, 1994).

All of these advantages allow the chloroplast genome to be a useful tool for evolutionary studies. The chloroplast gene encoding the large subunit of ribulose-1, 5-bisphosphate carboxylase/ oxygenase (*rbc*L) was used extensively in molecular systematic studies among angiosperms (Chase et al., 1993). Other chloroplast genes such as *atp*B, *ndh*F and *mat*K, were also applied (Soltis and Soltis, 1998; Judd et al., 1999). RFLP analysis of both chloroplast and ribosomal DNA sequences were also used to identify the parental origin of polyploid genomes with multiple origins (Soltis et al., 1992). Multiple origins of *Tragopogon miscellus* and *T. mirus* were studied using chloroplast and rRNA data (Soltis and Soltis, 1989; Soltis and Soltis, 1991).

All previous studies confirmed a maternal lineage of chloroplast inheritance among *Triticum* and *Aegilops* species (Ogihara and Tsunewaki, 1982; Murai and Tsunewaki, 1986; Tsunewaki, 1993; Wang et al., 1997). Genetic diversity in *Ae. triuncialis* was studied using the chloroplast genome. Results indicated its multiple origins from reciprocal crosses between *Ae. umbellulata* and *Ae. caudata* (Murai and Tsunewaki,1986). Ogihara and Tsunewaki (1988) studied chloroplast genomes of alloplasmic lines of 35 species of *Triticum* and *Aegilops* with 13 restriction enzymes. Their results showed 33 nucleotide substitutions distributed equally throughout the genome, in contrast to 14 insertion/deletion mutations (indels). Six of fourteen indels were located between the genes *rbc*L and *pet*A. The region was designated as a hotspot

and not only contained many direct and inverted repeats near the indel region but also was AT rich (Ogihara et al., 1992). They suggested that these two characteristics might be responsible for the mutation. In previous studies, *Ae. caudata*, *Ae. triuncialis* and synthetic *Ae. triuncialis* had chloroplast type 2 which included a 300 base pair deletion within the hotspot region. Unlike *Ae. caudata*, *Ae. umbellulata* which had chloroplast type 3 did not have this deletion (Ogihara and Tsunewaki, 1988). This deletion was one of many structural changes distinguishing between *Ae. caudata* and *Ae. umbellulata.* This region seemed promising for distinguishing these genomes; although, only one alloplasmic line per species was analyzed. The region was studied further using nucleotide sequence comparison among two alloplasmic lines of *Triticum. aestivum* cv. Chinese Spring carrying cytoplasm of *Ae. crassa* and *Ae. squarrosa* and one euplasmic line, *T. aestivum* cv. Chinese Spring. Ogihara et al. (1991) found that nucleotides at the intergenic regions diverged ten times faster than those of coding regions. Wang et al. (1997) concluded that the genetic relationship of the chloroplast genomes of the U genome of *Ae. umbellulata* and the C or D genomes of *Ae. caudata* and *Ae. squarrosa* are only moderately close.

Due to superior colonizing abilities of *Aegilops triuncialis* compared to its diploid ancestors, many questions still need to be answered. What is the level of genetic differentiation between the diploid parents? How much genetic variability is within *Aegilops triuncialis* relative to diploid progenitors? Was this genetic variability due to the genetic consequences of polyploidization? How frequently did this species form? Will

DNA sequence data from chloroplast and nuclear genomes give similar results? Soltis and Soltis (1993) stated that different types of DNA might lead to different conclusions.

Investigation of these questions was carried out to gain a better understanding of the evolutionary dynamics of *Ae. triuncialis*. Molecular genetic data were the approach selected as they provide a wealth of new insights into polyploid evolution. Moreover, molecular genetic approaches have provided critical data regarding the genetic consequences of polyploid evolution. From previous molecular results (Badaeva et al., 1996; Dvorak and Zhang, 1992), the U and C genomes appear to be closely related, such that coding regions are too similar to provide enough discrimination between *Ae. triuncialis* and its diploid progenitors. Therefore, noncoding regions, both in chloroplast and nuclear genomes, were selected to assess genetic diversity between *Ae. triuncialis* (UC genome) and its ancestors *Ae. umbellulata* (U genome) and *Ae. caudata* (C genome). First, we screened primers to identify those which amplify all three genomes to observe genetic variability among them. Second, genome specific primers for U, C and chloroplast genomes were selected to enhance data interpretation. Polyploid organisms receive nuclear genome from both maternal and paternal parents. It is difficult to identify the origin of specific alleles if more than one genome are amplified. Genome specific primers allow unambiguous genome assessment. Since DNA sequence comparison provides the greatest discrimination of evolutionary relationships, sequence data were obtained from DNA segments amplified using genome specific primers. Phylogenetic analysis based on DNA sequence data was performed to determine the relationships of

the diploid ancestors and the allotetraploid species and to address the possibility that the allopolyploid *Ae. triuncialis* formed multiple times.

Materials and Methods

Plant Materials

Three species of wild wheats were chosen. Thirty one accessions of *Aegilops caudata*, 33 accessions of *Aegilops umbellulata* and 212 accessions of *Aegilops triuncialis* were obtained from Harold E. Bockelman USDA National Small Grain Collection, Aberdeen, Idaho, USA. *Aegilops caudata* and *Aegilops umbellulata* are diploid progenitors of *Aegilops triuncialis*. Three accessions of *Aegilops caudata*, two accessions of *Aegilops umbellulata* and six accessions of *Aegilops triuncialis* were requested from Wheat Genetics Resource, Kansas State University. Four accessions of *Aegilops triuncialis* were obtained from Plant Germplasm Institute, Kyoto University Japan (http://www.shigen.nig.ac.jp/wheat/wheat.html?228,31) (Murai and Tsunewaki 1986). *Aegilops caudata* came from only Turkey and Greece. *Aegilops umbellulata* predominantly originated from Turkey. *Aegilops triuncialis* is widely distributed throughout the Mediterranean, Black Sea region, the Middle East of Asia and western Africa. *Triticum tauschii* accession KU2050 from Afghanistan was used as an outgroup species.

Genomic DNA Isolation

All plants were grown in the Plant Growth Center at Montana State University and young leaves were collected for total genomic DNA extraction (Riede and Anderson, 1996). A single plant was used per each accession. Young leaves, weighing between 20 and 300 mg, were collected and ground in liquid nitrogen using an electric drill and Kontes pestle in a 2 ml microcentrifuge tube. After grinding, 750 µl of prewarmed extraction buffer [0.5M NaCl, 0.1M Tris-HCl pH 8.0, 0.05 M ethylenediaminetetra-acetic acid (EDTA), 8.4 gm/L sodium dodecyl sulfate (SDS), 3.8 gm/L sodium bisulfite] was added and vortexed until suspended. Samples were incubated at 65 °C in a waterbath for 45 minutes and mixed by gentle inversion every ten minutes. After the incubation period, samples were cooled on ice and 750 µl of chloroform was added. Samples were mixed to homogeneity and centrifuged at 14,000 G for ten minutes. Approximately 600 µl of the upper phase was drawn off and transferred to a new tube containing 1 ml cold 95 % ethanol. DNA pellets were precipitated by gentle inversion followed by centrifugation at 14,000 G for ten minutes. Genomic DNA was washed with 1 ml 70 % ethanol, shaking at least 1 hour and followed by centrifugation at 14,000 G for four minutes. The supernatant was decanted. The DNA pellet was air dried and resuspended in 100 µl TE buffer. Genomic DNA sample were quantified by running 1 µl on a 1 % agarose gel with 1 X TBE buffer and comparing with a precision molecular mass standard (Bio-Rad®). The working concentrations of genomic DNA were then adjusted to approximately 100 ng/µl for use as template DNA in PCR reaction.

STS-PCR Primers for PCR Amplification

A total of 84 primer sets were used to screen accessions of *Ae. caudata*, *Ae. umbellulata* and *Ae. triuncialis*. The nucleotide primers were synthesized by Sigma Genosys, USA. Twenty-nine primer sets were developed from mapped RFLP clones of D genome diploid *Triticum tauschii* (Talbert et al., 1994). Thirty-two primer sets were designed in the chloroplast hotspot region. Nine and six primer sets were developed from wheat and barley genomic DNA, respectively. Six and two primer sets were developed from barley and oat cDNA(Tragoonrung et al., 1992). Some primers are shown in Table 1. The Cp6 (U6/R6) primer set was designed in this study to amplify the noncoding region between *ycf*4 and *cem*A genes within the chloroplast genome of *Triticum* and *Aegilops* species. The last four primers in the Table 1 were used for sequencing cloned inserts (see below).

PCR Amplification and Analysis

PCR amplifications were conducted in 50 µl reactions consisting of 1 X Promega reaction buffer, 200 µM of dNTPs, 1.5 mM $MgCl_2$, 400 nM of left and right primers, 0.75 U of Taq polymerase, and 100 ng of genomic DNA. The PCR cycle is shown in Table 2 (Talbert et al. 1994). Since the primers were derived from non target species, we chose lower annealing temperatures. The annealing temperature, a controlling factor for PCR amplification, will enhance or suppress artifact formation when it is decreased or increased (Watson, 1989). The PCR was performed in a PTC-100 programmable thermocycler (MJ Research, Inc.).

Table 1. Primers and sequence of primers for PCR amplification

Name of primer	Sequence	Location[a]	Genome specific
G43 Forward	5'-GGCGC ATGCA CCAAA ATGTT-3'	6D	U
G43 Reverse	5'-ACCTT GTCGT GCATA GGAAC-3'	6D	U
D21 Forward	5'-TCTTC CAGTT AGAGA TCTCC-3'	4D	C
D21 Reverse	5'-TCGTT CGTAC TAGTA GTACC-3'	4D	C
Cp6 Forward	5'-GCTGC CGAAT TGGCC TATTT-3'	Cp[b]	cp
Cp6 Reverse	5'-GCCTG GTATT CCACC AATTC-3'	cp	cp
D2 Forward	5'-CGAAT GTTTC TACTG CGCTG T-3'	7ABD	NA
D2 Reverse	5'-CTCCC TGTTT GTGGA AAGCT-3'	7ABD	NA
D15 Forward	5'-GTCTT CACGG AGATC TGTAT-3'	1D,5BD, 7BD	NA
D15 Reverse	5'-GCTGC CTGTT TTGTT TCGCA-3'	1D,5BD, 7BD	NA
F8 Forward	5'-GCATT ATCAT CAGCT GAAAG-3'	4ABD	NA
F8 Reverse	5'-GTTCA AGGCA GACCT TGACT-3'	4ABD	NA
G12 Forward	5'-CCAGT GTTGT AGTTC TCTAT-3'	2B,2D	U
G12 Reverse	5'-TATAC TTCTG AGCTG CCGAG-3'	2B,2D	U
WG232 Forward	5'-CCTCA GTGTT TCAGG GTAAA-3'	7A,4AD	NA
WG232 Reverse	5'-TGGAC TCGTG TTCAA TAATG-3'	7A,4AD	NA
M13 Forward	5'-GTAAA ACGAC GGCCA G -3'		
M13 Reverse	5'-CAGGA AACAG CTATG AC -3'		
SP6	5'-TATTT AGGTG ACACT ATAG-3'		
T7	5'-TAATA CGACT CACTA TAGGG-3'		

[a] Map location in wheat or *T. tauschii.*
[b] Chloroplast genome

Table 2. PCR parameters for DNA amplification

Segment	Step	Temperature (°C)	Time	Number of cycles
1	Denaturing	94	4 minutes	1
2	Denaturing Annealing Extension	94 45 or 50 72	1 minute 1 minute 1.2 minutes	30
3	Extension Holding Holding	72 4 10	7 minutes 4 minutes ∞	1 1 1

PCR Product Evaluation

The PCR products were analyzed on either 1 % agarose gel with 1 X Tris-borate EDTA running buffer or on 7 % polyacrylamide gel with 0.5 X Tris-borate EDTA running buffer. The gels were stained with ethidium bromide, visualized with UV light, and photographed.

Restriction Endonuclease Digestion

Some of the PCR products were digested with restriction endonucleases to observe differences. Restriction endonucleases are groups of enzymes that bind and cleave double-stranded DNA at specific sequences. Restriction digest contained approximately 1 U of restriction enzyme, 1 X reaction buffer and sterile distilled water to

give a total volume of 20 µl. The restriction enzymes used in this study including their restriction sequences and optimal temperatures are shown in Table 3. After one hour digestion had been completed, and the digested products are analyzed by 7 % polyacrylamide gel electrophoresis.

Table 3. Restriction endonucleases with their recognition sequences and optimal temperatures

Restriction enzyme	Recognition sequences	Optimal temperature (°C)
*Dde*I	C↓TNAG	37
*Eco*RI	G↓AATTC	37
*Hha*I	GCG↓C	37
*Hin*dIII	A↓AGCTT	37
*Hinf*I	G↓ANTC	37
*Mse*I	T↓TAA	37
*Rsa*I	GT↓AC	37
*Xba*I	T↓CTAGA	37

Diversity Analysis

The allelic diversity of nuclear and chloroplast genomes was calculated as indicated by the polymorphism information content (PIC) value described by Botstein et al. (1980) and modified by Anderson et al. (1993) for self-pollinated species. PIC value is the best indication for genetic diversity because it indicated the relative polymorphism value of each primer. The formulae is:

$$PIC_i = 1 - \sum_{j}^{n} p_{ij}^2$$

where p_{ij} is the frequency of the jth pattern of primer i and summation extends over n patterns.

Statistical Analysis

A χ^2 goodness-of-fit test was performed to test the nuclear and chloroplast correspondence for G43 and U6/R6 loci within *Ae. triuncialis*. A 2 x 2 two-way table was used for the χ^2 goodness-of-fit test. Significance was tested at the a = 0.05 level.

Cloning

PCR products were cloned to improve the quality of DNA sequencing. Ten µl of each PCR product was separated on 1 % agarose gel with Tris-borate EDTA running buffer. Band size was confirmed. Cloning for PCR product was accomplished using the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) or pGEM®-T vector (Promega, Madison, WI). Five to ten white colonies were selected from each plate and each single colony was cultured overnight in LB broth containing 50 µg/ml amplicillin. One µl of each culture was amplified by the same PCR condition as described previously. Twenty-five µl of each PCR product was analyzed on 1 % agarose gels with Tris-borate EDTA running buffer to ensure the insert was the correct size.

Plasmid DNA Preparation

Ten µl of each selected colony from previous procedure was cultured overnight in 3 ml of 2 X YT broth containing 50 µg/ml amplicillin at 37 °C and rotating 125 rpm. Three ml of the culture was collected and placed in two ml microcentrifuge tubes. One point five ml of cell culture was centrifuged at 12,000 G for 20 seconds twice at room temperature in the same microcentrifuge tube. The supernatant was discarded and the

bacterial pellet was resuspended in 200 µl cell suspension buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA) then mixed by vortexing. Two hundred µl of freshly prepared alkaline lysis solution (1 % SDS in 0.2 M NaOH) was added into each tube and mixed by inverting the tube several times. After that, 200 µl of 2.55 M potassium acetate, pH 4.8, was added to neutralize the suspension and gently mixed by inverting. The precipitate was visible at this point. The samples were centrifuged 12,000 G for 5 minutes. The supernatant was transferred to a new clean tube that contained 300 µl of Prep-A-Gene plasmid binding buffer (Bio-Rad). Sixty µl of resuspended Prep-A-Gene matrix was added to each tube with ten minutes shaking incubation. The well-mixed solution was transferred into a spin column, placed into a 1.5 ml microcentrifuge tube, and centrifuged 12,000 G for 30 seconds to remove the liquid phase. Five hundred µl of Prep-A-Gene washing buffer was added into each column and centrifuged 30 seconds. This wash step was performed twice. A dry centrifugation was performed at 12,000 G for 4 minutes to ensure that all wash buffer was eluted from the spin column. One hundred µl sterile deionized water was added to the spin column to elute the plasmid DNA into a new catch tube. Alcohol precipitation was performed after this step to concentrate the sample. Eleven point one µl of 3M sodium acetate pH 5.2 and 227.78 µl of 95 % ethanol were added, mixed and incubated one hour at –80 °C. The samples were centrifuged at 12,000 G for 10 minutes and then the supernatant was removed. The pellet was washed with 100 µl 80 % ethanol and centrifuged again. Finally, the samples were air dried and resuspended in 11 µl sterile deionized water. One µl of the sample was quantified by

running on a 1 % agarose gel and compared to the precision molecular mass standard (Bio-rad). The purified plasmid was then ready for sequencing.

DNA Sequencing

Standard PCR was performed in 50 µl reactions using the same condition as in PCR amplification except 0.2 µl of Taq polymerase was used per reaction and segment 2 of the PCR cycle was repeated 35 times. PCR products were cleaned by Qiagen columns to remove excess primers and salts followed by precipitation with 3 M sodium acetate pH 4.8 and 95 % ethanol. Sequencing was done on an ABI377 automated DNA sequencer and the Perkin Elmer BigDye™ sequencing reaction kit (PE Biosystems). Sequences were read in both the forward and reverse directions either using the original primer sets or plasmid primer sets. Some samples were sent out for sequencing using the same protocol at Washington State University (Pullman).

Reconstructed Phylogenetic tree

The sequences from the same primer set were first aligned by ALIGN (Scientific and Educational Software, 1989) followed by manual alignment to minimize gaps. The data were analyzed using the parsimony heuristic search, neighbor joining, and bootstrap parsimony of the Phylogeny Using Parsimony Analysis (PAUP*) program version 4.0beta8 for 32 bit Microsoft window (Swofford, 1998). The stepwise addition option was used to find the most parsimonious bootstrap trees. Bootstrap was performed by using the full heuristic search option of PAUP* to calculate the robustness of each

branch. The analysis was set with the following parameters: 100 bootstrap replicates (Felsenstein, 1985) with gaps treated as missing data, tree bisection-reconstruction branch swapping and random sequence addition. All characters were weighed equally. Bootstrap values indicated the percentage of time that resampling yielded the same clade. The goodness of fit statistic was determined to estimate reliability of each phylogenetic tree. Consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated (Kluge and Farris, 1969; Farris, 1989). Pairwise genetic distances were calculated using the kimura2-parameter option in PHYLIP version 3.572c (DNADIST program) (Felsenstein, 1997) and MEGA (Kumar et al. 1993). *Triticum tauschii* was used as an outgroup taxon.

Results and Discussion

A total of 84 primer sets were screened on at least two accessions of each of *Ae. umbellulata*, *Ae. caudata* and *Ae. triuncialis* in order to identify genome-specific primer sets. PCR products were amplified using 45 °C (71.43 %) and 50 °C (68.65 %) annealing temperatures (Table 4). Forty-six primer sets amplified all three genomes and only three primer sets were genome-specific. U genome-specific primers were G12 and G43 and primer set D21 was C genome-specific. All primer sets did not amplified in all accessions since they were derived from related species (*Triticum tauschii*, wheat and barley), and not target species (see chapter 3) (Vanichanon et al, 1999). For preliminary work of cereal genomes, it is necessary to use primer sets that were created from across cereal

species. Due to grass genome colinearity study, Bennetzen (1999) proposed that the grasses have a single genetic system with some limitation.

Table 4. Genome specificity of primers based on annealing temperature

Annealing temperature	Number of Primers				% of primers that amplified at least some accessions
	U &UC specific	C & UC specific	All three genomes	Total	
45 °C	3	1	46	68	71.43%
50 °C	2	1	35	84	68.65%

Diversity in Polyploid *Aegilops triuncialis* and Diploid Progenitors

Six primer sets shown in Table 5 were selected to amplify PCR products from all 276 accessions of the three species to study genetic diversity. PIC values (Table 5) revealed that *Ae. caudata* was a more diverse species than *Ae. umbellulata* as reported by Chee et al. (1995). Average PIC values from all four nuclear primers (D2, D15, F8 and WG232) are 0.34, 0.84 and 0.62 for the U, C and UC genomes, respectively. These PIC values illustrated that *Ae. caudata* had the greatest diversity. *Ae. triuncialis* had more diversity than *Ae. umbellulata*, perhaps because of genetic diversity introduced from *Ae. caudata*. For example, the polymorphism seen in *Ae. triuncialis* using primer set D2 may

Table 5. Polymorphism restriction fragments distribution among U, C and UC genomes as a measure of genetic diversity.

Primer name/ Restrition Enzyme	Number of unique patterns (Number of accessions analyzed)[c]			Percentage of the most common pattern (%) observed in the U, C and UC genomes			Percentage of the most common pattern (%) observed in the U, C and UC genomes			PIC value		
	U	C	UC	U	C	UC	U	C	UC	U	C	UC
A. Nuclear Primers												
D2/ *Rsa*I	5 (34)	4 (29)	5 (99)	85.3	3.5	0	2.9	51.7	44.4	0.28	0.70	0.66
D15/ *Hha*I	6 (30)	6 (8)	5 (155)	67.6	9.1	71.6	67.6[a]	9.1[a]	71.6[a]	0.53	0.99	0.50
F8/ *Hinf*I	2 (30)	3 (20)	12 (70)	85.3	4.8	11.4	0	54.6	0	0.28	0.71	0.85
WG232/ *Mse*I	4 (30)	3 (12)	2 (170)	85.0	6.1	89.4	0	21.2	8.7	0.28	0.94	0.46
Average PIC value for all nuclear primers										**0.34**	**0.84**	**0.62**
Standard deviation of PIC value for all nuclear primers										0.13	0.15	0.17
B. Genome Specific Primers												
U specific G43/ *Dde*I	2 (28)	- (33)	2 (185)	52.9	-[b]	54.1	-	-	-	0.64	-	0.59
Chloroplast U6/R6/ NA	2 (33)	2 (27)	2 (203)	88.2	12.1	50.7	8.8	69.7	51.2	0.22	0.41	0.48

[a] There was the same bandind pattern in U diploids

[b] G43 did not amplify any products in the C genome accessions tested

[c] A total number of 34, 33 and 220 accessions of *Ae. umbellulata, Ae.caudata* and *Ae. triuncialis*, respectively, were analyzed

have originated from *Ae. caudata* because the most common pattern in *Ae. caudata* was also common in *Ae. triuncialis* (Table 5). Depending on the primer sets, *Ae. triuncialis* had similar variability to either *Ae. umbellulata* (D15) or *Ae. caudata* (D2). For instance, primer set D15 showed six banding patterns within *Ae. umbellulata* and 67.6 % of the 34 accessions had a common pattern. This pattern was also seen in 71.6 % of *Ae. triuncialis* (209 accessions). Only four accessions of *Ae. triuncialis* showed different banding patterns. The genetic variation within the allotetraploid may result from the genetic variation introduced from the diploid progenitors through multiple polyploidization or introgression or genetic variation that accumulated by mutation after polyploidization. It appears that polyploid *Ae. triuncialis* have less variation than diploid progenitor. PIC values from D15 were calculated 0.53, 0.50 and 0.99 for the U, UC and C genomes, respectively. From the D15 result, polyploidization event may occur only a few times resulting in less variation in the UC genomes.

Multiple Origins of *Aegilops triuncialis*
Inferred Using Nuclear DNA Sequence Analysis

Nuclear DNA analysis was chosen to assess multiple origins of polyploid wild wheat. Genome-specific primers were preferred because they provided unambiguous evidence that a specific *Ae. triuncialis* pattern came from either *Ae. umbellulata* or *Ae. caudata*. Therefore, G43 was selected for further evaluation because it was a U genome-specific primer. *Dde*I restriction digestion of the G43 amplified product yielded two banding patterns: allele A (Figure 1 lane U05, U"C10; fragment sizes: 300, 240, 220 base pairs) and allele B (Figure 1 lane U08, UC.04; fragment sizes: 240, 220, 200, 100 base

pairs). Both alleles were observed in both *Ae. umbellulata* and *Ae. triuncialis*. This is evidence that *Ae. triuncialis* inherited two distinct alleles from *Ae. umbellulata*, indicating that at least two distinct polyploidization events occurred.

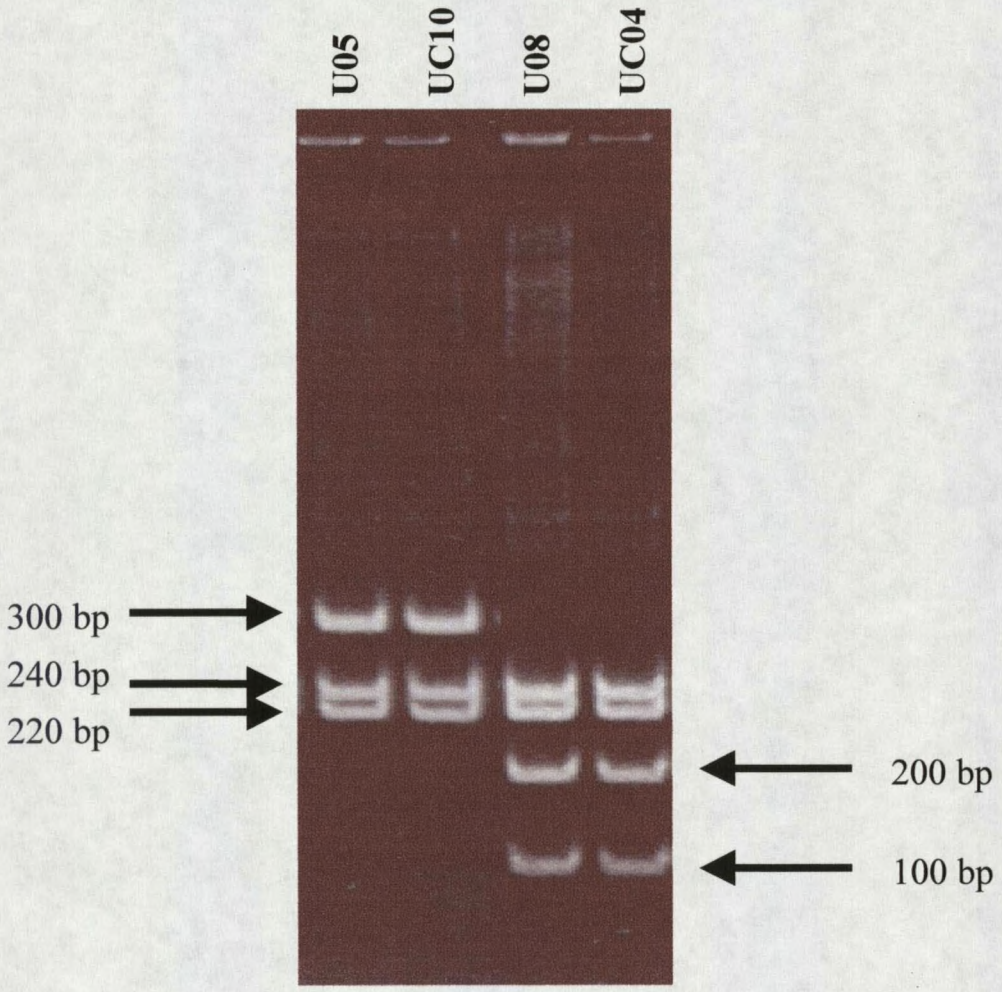


Figure 1. *Dde*I-digested DNA amplified from *Ae. umbellulata* and *Ae. triuncialis* using primer set G43. Lanes: U5 and UC10 are A alelle. U8 and UC4 are B allele.

Plant accessions from six different locations were analyzed with G43 primer set (Table 6). There was only one accession of *Ae. umbellulata* in each location and each had

Table 6. G43 alleles of sympatric accessions in six locations

Location	G43 locus	
	A allele	B allele
Turkey, Ankara	**U 02** (*Ae. umbellulata*)	UC35
	UC88	UC36
	UC102	UC81
	UC195	
Turkey, Canakkale	**U13** (*Ae. umbellulata*)	UC189
	UC10	UC190
	UC26	UC196
	UC187	
	UC188	
Turkey, Siirt	**U35** (*Ae. umbellulata*)	UC181
Turkey, Usak	**U20** (*Ae. umbellulata*)	UC123
	UC124	
Syria	**U10** (*Ae. umbellulata*)	UC03
	UC104	UC105
	UC109	UC107
		UC108
Yugoslavia, Serbia	**U01** (*Ae. umbellulata*)	UC04
	UC68	UC67
	UC79	UC72

the A allele. Sympatric accessions of *Ae. triuncialis* had both alleles, except at one location, Siirt Turkey, one *Ae. triuncialis* accession had only the B allele. This result suggested that hybridization among *Ae. triuncialis* in the neighborhood location or

incomplete sampling in *Ae. umbellulata*. Chee et al. (1995) observed that a low level of introgression among tetraploid species occurred based on experiments with low copy DNA sequences.

A single nucleotide change detected by restriction fragment analysis is not sufficient for determining evolutionary relationships in that independent mutations may give rise to the same polymorphism. Additionally, products amplified by the same primer set may or may not be allelic (Erpelding et al., 1996). Because of limited data from the restriction fragment studies, the G43 locus was selected for comparative DNA sequence analysis to reveal more polymorphism and confirm allelism. We sequenced G43 alleles from 10 accessions of *Ae. umbellulata* and 8 accessions of *Ae. triuncialis*. A total of 802 bases of DNA sequence were obtained for all alleles. Sequence data confirmed the assumption from restriction analysis that both *Ae. umbellulata* and *Ae. triuncialis* bear the same two distinct alleles. Table 7 tabulates nucleotide differences that distinguish the two alleles. A total of 17 nucleotide substitutions characterized allele A from allele B in *Ae. umbellulata* and *Ae. triuncialis*. We assumed that allele B in *Ae. triuncialis* accessions was inherited from allele B in *Ae. umbellulata* accessions either paternally or maternally and similarly for allele A. Average genetic distances among the alleles from *Ae. umbellulata* and *Ae. triuncialis* were 0.0242 and 0.0124 respectively, indicating that genetic diversity among *Ae. umbellulata* alleles was twice as much as that among *Ae. triuncialis* alleles. Thus the polyploidization events did not completely sample all *Ae. umbellulata* alleles in the formation of *Ae. triuncialis*.

Table 7. Polymorphic bases which distinguish G43 alleles.

Site number	base pair number	Allele A		Allele B	
		U	UC	U	UC
1	7	T	T	A	A
2	17	G	G	T	T
3	107	A	A	G	G
4	305	T	T	-	-
5	306	C	C	-	-
6	307	G	G	-	-
7	309	C	C	T	T
8	320	T	T	C	C
9	358	C	C	T	T
10	361	C	C	T	T
11	365	C	C	A	A
12	386	A	A	G	G
13	389	A	A	G	G
14	410	G	G	C	C
15	411	-	-	T	T
16	412	-	-	A	A
17	413	-	-	A	A

Less diversity within *Ae. triuncialis* may be due to a close genetic relationship between the diploid progenitors. Based on the study of the synthetic *Brassica*, Song et al. (1995) concluded that the more diverse the parents, the more changes within allopolyploid plant directly after the polyploidization event. Conversely, another study of Ogihara and Tsunewaki (1988) stated that most chloroplast genomes differentiated at the diploid level and have not changed considerably after polyploidization in *Triticum* and *Aegilops* species. In our study, all *Ae. caudata* accessions did not amplify a product with G43 primer set. This may be due to major mutations within primer sequences in *Ae. caudata*. The multiple alignment of G43 sequences revealed 36 parsimony sites and 97 variable sites within 10 accessions of *Ae. umbellulata*. There were 28 parsimony sites and 35 variable sites within 8 accessions of *Ae. triuncialis*.

A PAUP-generated phylogenetic tree (Figure 2) showed that the U genome had more mutation in *Ae. umbellulata* than in *Ae. triuncialis*; in other words, the U genome in *Ae. triuncialis* was stable after the polyploidization event. Minimal tree length was 86. Goodness of fit statistics were calculated. Consistency, retention and rescaled consistency indices were 0.9186, 0.9517 and 0.8743, respectively. At node 26, bootstrap value equaled 100 % of resampling split into A and B allele groups (Figure 2). The group with A allele contained both *Ae. umbellulata* and *Ae. triuncialis*, as did the B allele group. *Triticum tauschii*, an outgroup, had the longest branch length.

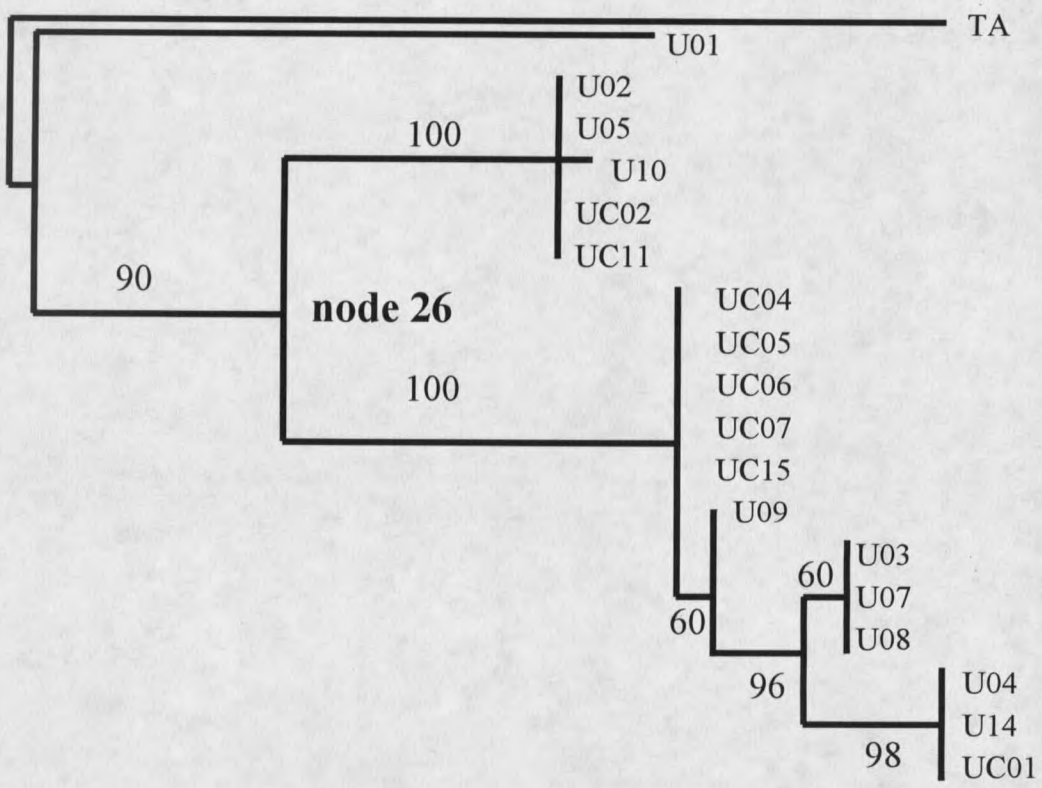


Figure 2. Maximum parsimony of G43 locus derived from heuristic search with a length of 86, CI of 0.9186, RI of 0.9517 and RC of 0.8743.Bootstrap values are given about each node. Species designation are given in figure.

All G43 outcomes indicated that polyploidization occurred at least twice and mutation rate after the events was low. All polymorphisms in the polyploid were also in the diploid progenitor. The U genome in *Ae. umbellulata* had more diversity, presumably because it was older or not all alelles were involved in polyploidization event.

Products amplified by primer set G12, also U genome-specific, were sequenced. A total of 201 bases showed no differences among 6 accessions of *Ae. umbellulata* and 10 accessions of *Ae. triuncialis* (Figure 3). Some regions of DNA in the U and UC

genomes had identical sequences, according to their close relationship, making these regions unusable for this study.

TGACTACTAATATGACTGCGGTCCT
CCGCCTACCGCTTAACGGGATGGAC
CGGGCAACGTACGCCAAGGCGAGAC
CTGCCTCTCCTCTTAAGTTGATTGG
TTTCTCTTTCTGCATTCCTTTTCTA
TGGTCTCATTTGGCATTTCTCCTTC
ATGATTATTATGTTGTCCCTTTTGG
GTTATCAGATGCTTTACCTCGGCAG
CTCAGAGTAT

Figure 3. DNA sequences of U2 accession in G12 locus

A third nuclear primer set, C genome-specific D21, was investigated. PCR products from this primer had the same size band (~1100 base pairs) in *Ae. caudata* and *Ae. triuncialis* (Figure 4). No different banding patterns were seen within or among *Ae. caudata* or *Ae. triuncialis* following the restriction fragment analysis. Nonetheless, DNA from the 5 accessions of *Ae. caudata* and the 11 accessions of *Ae. triuncialis* were amplified by D21, and products were cloned and sequenced. A total of 1,082 bases showed 6 parsimony sites and 26 variable sites. The C genome in *Ae. caudata* had more mutations. There were not enough parsimony sites to reconstruct a meaningful phylogenetic tree.

A BLAST search of sequences from three genome-specific primer sets revealed that no interesting DNA regions matched (http://www.ncbi.nlm.gov/blast/Blast.cgi).

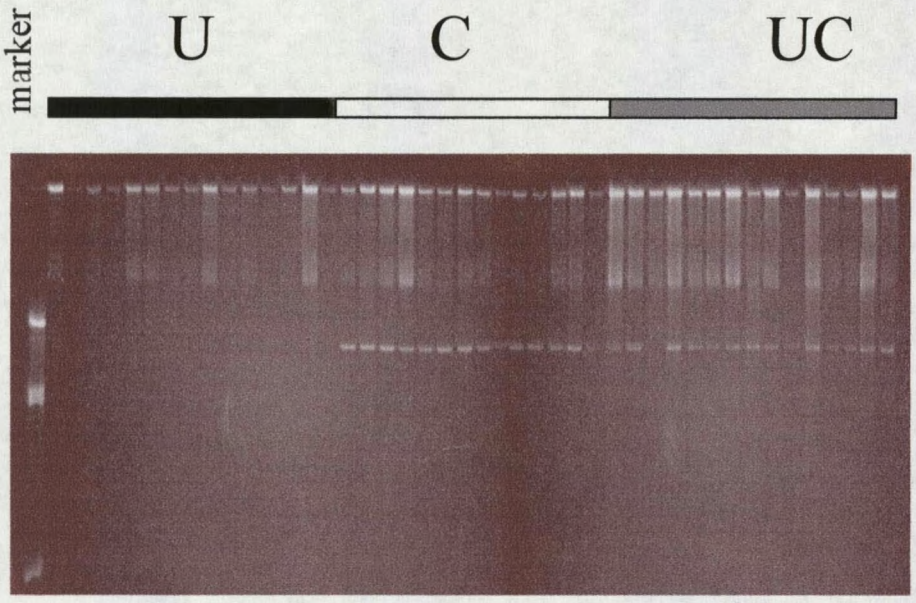


Figure 4. Gel picture of primer set D21. No band was amplified in *Ae. umbellulata* (U). The same band size was amplified in *Ae. caudata* (C) and *Ae. triuncialis* (UC). The marker contains three bands: 1769, 676 and 241 bp.

Multiple Origins of *Aegilops triuncialis*
Inferred Using Chloroplast DNA Sequence Analysis

In contrast to the nuclear genome, the chloroplast genome is inherited only through the maternal parent in *Triticum* and *Aegilops* species (Tsunewaki, 1993; Ogihara and Tsunewaki, 1988). Murai and Tsunewaki (1986) revealed that thirteen accessions of *Ae. triuncialis* had the type 2 chloroplast genome from *Ae. caudata* while eight accessions of *Ae. triuncialis* contained the type 3 chloroplast genome from *Ae. umbellulata*. Ogihara and Tsunewaki (1988) found that nucleus-cytoplasm hybrids or

alloplasmic lines of wheat (code number 02, 38 and 27) bearing *Ae. caudata, Ae. triuncialis* and synthetic *Ae. triuncialis* [an ampliploid between *Ae. caudata* (female parent) and *Ae. umbellulata* produced by Kondo in 1941] chloroplast genomes had chloroplast type 2 which included a 300 base deletion within the hotspot region. Alloplasmic lines (code number 03 and 26) having *Ae. umbellulata* and *Ae. triuncialis* chloroplast genomes had type 3 without deletion. The 300 base deletion within the hotspot region that might be specific to the C genome was investigated in this study.

We designed six primers on each side of the hot spot region in the chloroplast DNA and tested them in all combinations to identify the most reliable pair. Primer set U6/R6 amplified a 600 base intergenic region between *ycf*4 and *cem*A including the 300 base deletion found in *Ae. caudata* and no deletion in *Ae. umbellulata* (Ogihara and Tsunewaki, 1988). PCR analysis results showed that 23 of the *Ae. caudata* 33 accessions had the deletion (nucleotides inside the blue box in figure 5) and four of the 33 accessions lacked the deletion. Thirty of 34 accessions of *Ae. umbellulata* had no deletion but three of them had the deletion. This showed that not all C genomes had the deletion, so this deletion cannot distinguish the C genome apart from the U genome.

PCR data showed that some U chloroplast genome shared the deletion with the C genome. To determine additional polymorphism between Ae. *caudata* and *Ae. umbellulata* chloroplast genomes, we sequenced U6/R6 alleles: 10 accessions of *Ae. umbellulata* and four accessions of *Ae. caudata* lacking the deletion. All 10 accessions of *Ae. umbellulata* had nucleotide T at position 58329 in complete sequence of wheat chloroplast DNA, representing the intergenic region between *ycf*4 and *cem*A (Ogihara

GCTGCCGAATTGGCCTATTT CTTGCGCGTA CCAATTGAAG TATTTTGAGG

TATCTTTTTT GAACTGAGTT GAATGAAGAA AAGAAGAATT GG***AAGAAGAA***

AA**A**TTTTCTC AACACGGGGA GGAAGTCCCT TCTAAATTGG ATTTGTTATT

GTAAG**T**GGAT TTTTAAGTAT TTATCTAAAG GAAGGAACAA ACGAGGATAA

GAGAAATTTG CTTTTAATTT TTTTTTATCC AAGTGAGATA TATCGCATAC

TATTCTTCCT TTTTCATCCG AAAGGGCTTT TTTTTTATTC TATTTCACTA

TTTCATTCCA TCTAGATCTA AGAAAGAACC CAATGCACTG AAATTCCAC**A**

AATAACTAAT ATACAA***AA*** ***GAAGAA***TAGA TACAGGGTAT CAAACCTATA

GAGTTTTTGC TTCAAAGAAA TAGAAATATC ATGAAATAGA AATATCATCA

TATAGAGTCA GGGAATGGAA TAGAGTCAGC GAATGAAGCA TATTCATTAA

CAACTCCATT TACAGATCAA AAATGAAAAA AAAGAAAGCA TTGCCTTCTT

TACTATATCT TGTATTTATC GTACTTTTGC CTTGGGGGGT CTCTTCCTCA

TTTAACAAAT GTCTGGAACT TTGGATTAA GAATTGGTGGAATACCAGGC

Figure 5. DNA sequences of intergenic region between *ycf*4 and *cem*A in chloroplast genome from *Ae. umbellulata* (Clae 66). The boxed region indicates the deletion that occurs in some *Aegilops* chloroplasts. Bold italicized nucleotides represent a pair of directed repeats. Bold double underlined nucleotide demonstrates G/T transversion at position 58329. Bold single underlined nucleotides show additional polymorphisms at positions 58276 and 58523 that are associated with the G/T transversion at position 58329. Underlined nucleotides are left and right primers in *ycf*4 and *cem*A genes, respectively.

and Tsunewaki, 2000). All four accessions of *Ae. caudata* had nucleotide G at the same position (red and underline nucleotide in Figure 5). This nucleotide position was presumably genome-specific. Transversion mutation that occurred in this position might

reveal that nucleotide T is specific for *Ae. umbellulata* and nucleotide G is specific for *Ae. caudata.*

The fact that *Ae. triuncialis* had two chloroplast types, i.e. with and without the deletion, indicated at least two independent origins. Based on 14 accessions of *Ae. triuncialis* without the deletion in this region, seven of them had nucleotide G, indicating that had the diploid C genome maternal parent (one origin). Seven accessions had nucleotide T at the same position, indicating that had the diploid U genome maternal parent (another origin). For all accessions with deletion, at least one origin was assumed according to sequence data, no polymorphism was observed. From this data, we concluded that at least three origins occurred in formation of *Ae. triuncialis.*

We also sequenced an entire U6/R6 locus from five accessions of *Ae. caudata,* 12 accessions of *Ae. umbellulata* and 10 of *Ae. triuncialis* for more polymorphism. A total of 651 bases of DNA sequences were obtained for all alleles. Six parsimony sites, 20 variable sites and two indels were obtained. Data cannot be used to reconstruct a meaningful phylogenetic tree because of insufficient polymorphism. From the sequence, a pair of direct repeats were found flanking the deletion region (underline letters in Figure 5). The pair of direct repeats might be responsible for deletion mechanism (Ogihara et al., 1988, 1992).

Linkage Disequilibrium between
Nuclear and Chloroplast Sequences

Nuclear and chloroplast polymorphisms illustrated linkage disequilibrium because of nuclear and chloroplast correspondence based on $\chi^2 = 6.859$; 3 df; $P = 0.0765$ (Table

8). The U6/R6 chloroplast type with the deletion was more often associated with the G43 B allele (61 accessions) than would be expected (52.39) if these alleles were independent. The most plausible explanation is the U6/R6 (with deletion) chloroplast and

Table 8. Number of *Ae. triuncialis* accessions that have G43 & U6/R6 alleles

U6/R6	G43		Total
	A allele	B allele	
No deletion	47 [38.3937]	55 [63.6189]	102 (0.5484)
Deletion	23 [31.6167]	61 [52.3893]	84 (0.4516)
Total	70 (0.3764)	116 (0.6237)	186

[ ] Expected value
( ) Genotype frequency
$\chi 2 = 6.859$; 3 *df*; at $P = 0.0765$

the G43 B allele were introduced from a single U parent. Although hybridization has occurred among accessions of independent origin, it has not been sufficient to cause linkage equilibrium among the chloroplast and nuclear sequences. None of *Ae. umbellulata* accessions had both the deletion for the U6/R6 locus and the A allele for the G43 locus (Table 9) while 23 accessions of *Ae. triuncialis* had both. Since *Ae. umbellulata* was supposed to be a maternal parent for all *Ae. triuncialis ssp. triuncialis* (Slageren, 1994), *Ae. triuncialis ssp. triuncialis* should not have both loci. One possible mechanism for the appearance of these loci in *ssp. triuncialis* is hybridization among *Ae.*

triuncialis ssp. triuncialis or between two subspecies of *Ae. triuncialis* that independently formed. which is that some *ssp. triuncialis* might have *Ae. caudata* as the maternal parent. This possibility confirmed by our chloroplast results showing some accessions of *Ae. triuncialis ssp. triuncialis* (UC3, UC12, UC40, UC 42, UC48, UC50 and UC200) have *Ae. caudata* as a maternal parent if the genome-specific nucleotide was correct.

Table 9. Number of *Ae. umbellulata* accessions that have G43 & U6/R6 alleles

U6/R6	G43		Total
	A allele	B allele	
No deletion	10	16	26
Deletion	0	2	2
Total	10	18	28

The morphological trait anther length is an important character in the tribe Triticeae for determining breeding system. *Ae. caudata* is mainly outcrossing with a maximum anther length of 7.08 mm whereas *Ae. umbellulata* has a maximum anther length of 3.10 mm and is mainly inbreeding (Hammer and Matzk; 1993). Therefore, homozygotes are mainly evident in the U genome diploid species. As mention before that *Ae. caudata* is abundant throughout its range and sheds more pollen according to anther length; therefore, *Ae. caudata* may usually serve as a paternal parent of *Ae. triuncialis* in nature. The anther length is the one plausible reason that *Ae. umbellulata* is hypothesized

to function as a maternal parent. Therefore, *Ae. triuncialis ssp. persica* happens to be rare throughout its range.

Furthermore, eleven accessions of *Ae. triuncialis ssp. persica* were studied. Only one of them (UC P11) had both allele A for G43 locus and the deletion for U6/R6 locus. Four accessions had only the deletion for U6/R6 locus and no amplification for G43. Six accessions did not amplify either locus. These results may imply that in the formation of *Ae. triuncialis ssp. persica* dramatic changes occurred in the U genome and the chloroplast of the C genome because of nuclear-cytoplasm interaction. However, lack of amplification of several accessions makes any conclusions tenuous. On the contrary, when the U genome served as a maternal progenitor of *Ae. triuncialis ssp. triuncialis*, nuclear genome from the C diploid did not change intensely.

In conclusion, our results suggest that at least three polyploidization events occurred to form Ae. *triuncialis*, allotetraploid wild wheat. These multiple origins might lead to genetic and ecological advantages that contribute to the success of polyploid organism. Due to the less genetic diversity in allotetraploid species compared to each diploid progenitors, we conclude that there are few changes after polyploidization. *Ae. truncialis* has provided an good example for multiple origins of *Triticum* and *Aegilops* polyploid species with less genetic consequences. There are remaining questions related to this allotetraploid species, (1) why is *Ae. triuncialis ssp. triuncialis* so successful in a wide variety of different environments compared *to Ae. triuncialis ssp. persica*? (2) Do both species undergo hybridization between themselves? (3) How frequently? (4) What is the fate of the offspring?

CHAPTER 3

PROPERTIES OF SEQUENCE-TAGGED-SITE PRIMER SETS INFLUENCING REPEATIBILITY

Introduction

The polymerase chain reaction (PCR) is widely used in plant genetics, including in map construction, tagging specific genes, and more recently, genomics approaches to gene identification. PCR has become the standard procedure in plant molecular biology because of efficiency, ease and versatility. One advantage of PCR is that primer sequences can be shared and easily synthesized, obviating the need for exchange between labs of biological materials as required with clones for restriction fragment length polymorphism. However, a frequent observation has been that results from a particular primer pair may vary between laboratories (Linz, 1990; He et al., 1994). That is, a primer pair that produces a product marking a particular chromosome region for one lab may not produce the same product when the experiment is repeated in another laboratory. This limits the utility of sharing primer sequences among labs.

The relationship of the primer to the template sequence influences reproducibility of PCR reactions. For instance, high specificity of the primer to the target sequence decreases mis-priming and resultant amplification of extraneous DNA (Rychlik and Rhoads, 1989). This factor may be especially important in the cereals, where primer sets are often transferred

across cereal species due to conservation of map order (Erpelding et al., 1996). An overall GC content near 50% has been considered desirable since higher GC content may result in mis-priming due to high stability of imperfectly matched primer-template complexes (Kim and Smithies, 1988; Innis and Gelfand, 1990; Dieffenbach et al., 1993). Excessive T_m difference between primers and the targeted product can lead to low product yield (Innis and Gelfand, 1990).

In addition to primer-template relationships, internal characteristics of single primers, and the relationship between primers in a set, may influence repeatability. The melting temperature (T_m) of the left and right primers of a pair should be similar to avoid nonspecific amplification (Kim and Smithies, 1988; Dieffenbach et al., 1993). Primers should have low internal stability at the 3'-end so that false priming due to base pairing with non-target sequences is lessened (Rychlik, 1995). Internal complementary within a single primer enhances hairpin loop formation and reduces the annealing of the primer to the target sequence (Rychlik and Rhoads, 1989).

Complementarity between primers can give rise to primer-dimer formation, which in turn gives rise to artifactual bands. The possibility of primer-dimer formation with a primer and itself (intra-primer) or between the left and right primers of a set (inter-primer) is dependent on more than one factor. Dimer formation can only occur if there are complementary base pairings between strands, though Watson (1989) showed that a single base pair at the 3'-terminus can be sufficient for dimerization. Additionally, dimer formation is enhanced if one primer in the potential dimer has a 3'-overhang which can serve as a

template for extension by *Taq* polymerase. Brownie et al. (1997) concluded that inter-primer dimerization would decrease target yield more than intra-primer interactions.

Genomics approaches will accelerate the number of available PCR-based markers for plant genetics studies. For instance, expressed sequence tag (EST) libraries promise thousands of unique sequence markers (Rounsley et al., 1996). Many applications of the markers will require PCR. There have been few empirical studies regarding characteristics important in repeatability of primer sets. Researchers have developed several hundred sequence-tagged-site (STS) PCR primer sets from wheat and barley (Tragoonrung et al., 1992; Talbert et al., 1994; Blake et al., 1996). Primer sets have been sent to more than one hundred cooperators around the world, and results have been mixed as to their reliability. All primer sequences were designed targeting attributes such as unlikely primer-dimer formation, 50% GC content, and low potential for hairpin loop formation. However, there is considerable variation among the primer sets for these characteristics. For this report, we used 96 previously designed STS primer sets to identify factors which influence primer set reliability.

Materials and Methods

Materials

A total of 96 primer sets were analyzed, with 61 designed from genomic DNA sequences and 35 designed from cDNA sequences. A total of 28, 9, and 24 primer sets were from *Triticum tauschii*, wheat and barley genomic DNA, respectively. A total of 10, 21, and

4 primer sets were from wheat, barley, and oat cDNA, respectively. DNA was extracted from four genotypes using the method of Dellaporta et al. (1988), including two wheat samples (varieties Hi-Line and Chinese Spring), and two barley samples (varieties Chinook and Steptoe). For a subset of the primers sets, repeatability was tested on multiple DNA isolations for Chinese Spring and Hi-Line.

PCR Protocol

PCR reaction mixtures and thermocycler programs were performed as detailed previously with a standard annealing temperature of 50 °C for all primers (Talbert et al., 1994). PCR reaction mixtures of 200 µl were made for each primer/genotype combination and 50 µl aliquots were run in four thermocyclers from different manufacturers (Coy, Grass Lake, MI; MJ Research, Inc., Watertown, MA; Perkin Elmer, Norwalk, CT; and Barnstead/Thermolyne, Dubuque, IA). PCR products of a single primer pair from all four genotypes and thermocyclers were electrophoresized on 7 % acrylamide gels and stained with ethidium bromide. The reactions were repeated with a subset of 12 primer sets with the annealing temperature raised to 55 °C.

Scoring Bands

PCR products for a particular primer set were subjectively classified as major or minor bands, based on band intensity. The number of bands, both major and minor were recorded for each primer set by counting the maximum number of bands amplified among the four thermocyclers. Primer repeatability was scored separately for major and minor bands. A primer set was judged repeatable within a genotype if all major (or minor) bands were

amplified in all four thermocyclers at nearly equal intensity (Figure 6). If a primer failed to amplify the same products in one or more thermocyclers it was classified as non-repeatable (Figure 7).

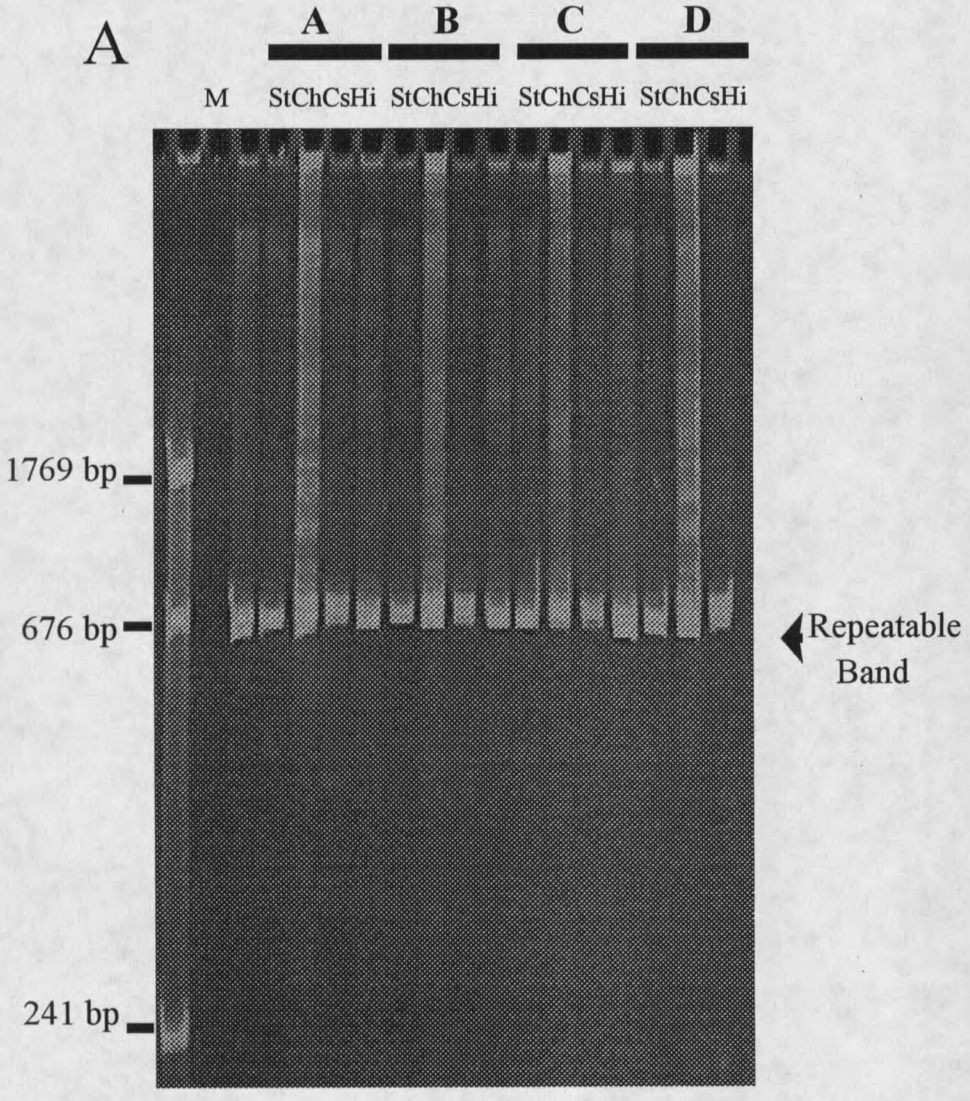


Figure 6. PCR products amplified from Steptoe (St), Chinook (Ch), Chinese Spring (Cs) and Hi- Line (Hi) DNA. M indicates molecular weight marker. A, B, C, and D represent the four thermocyclers used in the study. Amplification with primer set ABG601 of a single product, repeatable across all thermocyclers and genotypes.

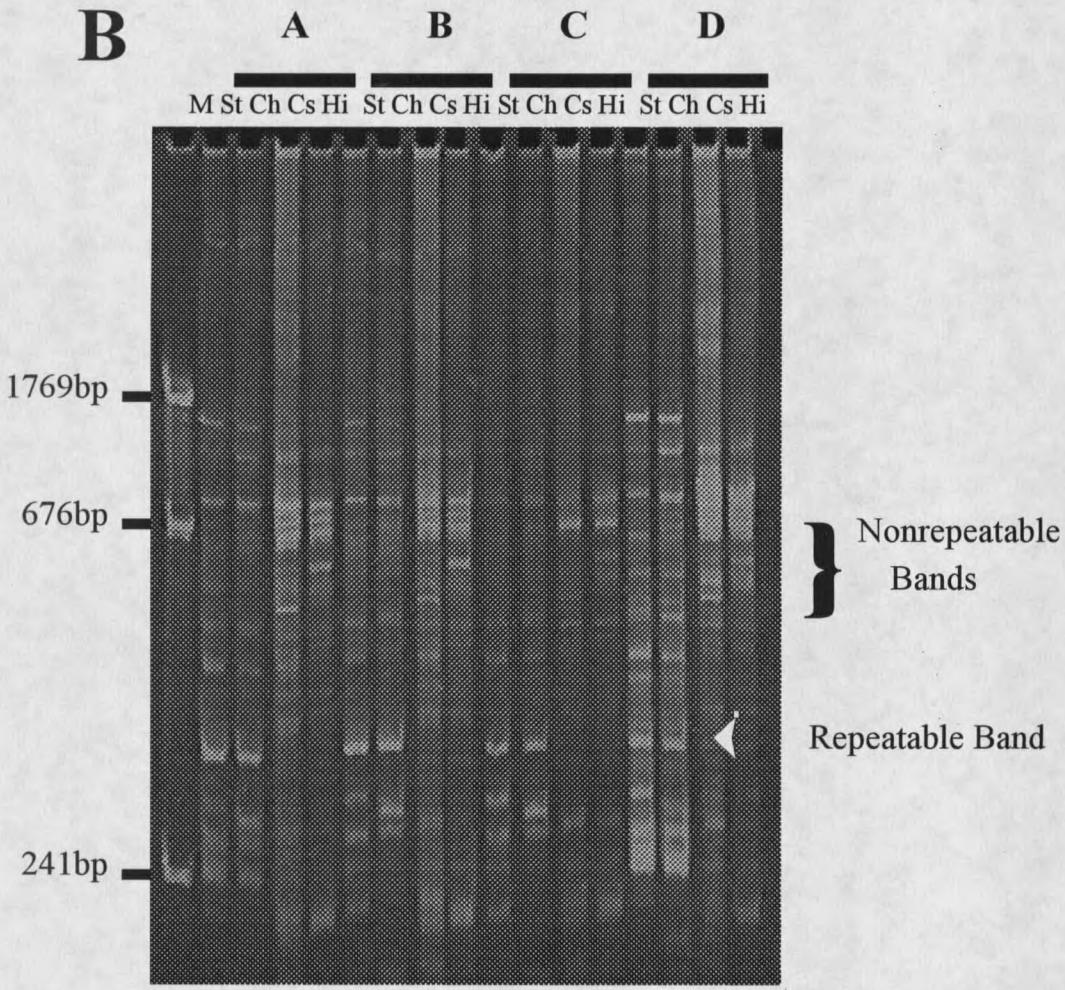


Figure 7. PCR products amplified from Steptoe (St), Chinook (Ch), Chinese Spring (Cs), and Hi- Line (Hi) DNA. M indicates molecular weight marker. A, B, C, and D represent the four thermocyclers used in the study. Amplification with primer set ABG317. Both repeatable and non-repeatable bands are seen depending on the genotype.

Primer Characteristics

Primers were evaluated for six characteristics as described in Table 10. Overall mean and standard deviation are given for each characteristic. Additionally, primer sets were categorized for primer source (i.e. primers derived from either barley, oats or wheat, including

T. tauschii) and primer type (i.e. primers derived from cDNA or genomic DNA clones). Certain characteristics were determined separately for the left and right primers of each pair: GC content, primer-dimer formation, hairpin loop stability and 3'-end stability. Other characteristics were features of the primer pair: primer type, primer source, primer-dimer formation, the melting temperature difference between the left and right primers (T_m difference), and the difference between the primer with the highest melting temperature in a primer set and the annealing temperature (T_m -T_{ann}).

The characteristic, T_m -T_{ann}, was used in lieu of the difference between the product T_m and the primer T_m. Almost all of the primers used were designed from only partial sequence of their respective clone, making it impossible to determine the product T_m. Stability of the 3'-end and hairpin loop formation were scored based on free energy values (ΔG). T_m and ΔG values were determined by the OLIGO program (Rychlik, 1992). Primer-dimer formation was measured with a composite score of two factors. The number of complementary base matches minus the number of mismatches between complementary bases was added to the length (number of bases) of any 3'-terminal overhang of the potential primer-dimer. If the number of complementary base matches minus the number of mismatches was 2 or less, then any 3'-terminal overhang was discounted and the potential primer-dimer was given a score of 0. Primer length (number of bases) was not used in this analysis as there was little variation among primers.

Statistical Analysis

Primer sets were classified as repeatable or not repeatable for major and minor bands for each genotype. Primer repeatability and characteristics were measured for all primers. Analysis of variance was used to determine primer characteristics that differed between repeatable versus non-repeatable primer sets. Significant differences between repeatable and non-repeatable means were tested using a F test.

A χ^2 goodness of fit test was performed to test the independence of genotype and repeatability for major and minor bands. Primer source and primer type were also tested for independence from repeatability for major and minor bands, requiring a further classification of the data. Primers were classified as derived from the target species, designated as "within genera" (i.e. barley-derived primers amplifying barley genotypes) or not derived from the target species, designated as "outside genera" (i.e. barley-derived primers amplifying wheat genotypes) for each genotype. Classified data was placed into a 2 x 2 two-way table for the χ^2 goodness of fit test. Significance was tested at the 0.05 level.

Results and Discussion

A total of 96 primer sets were tested on two wheat and two barley genomic DNA samples, using four different thermocyclers. Reaction conditions were programmed identically for all four thermocyclers. All thermocyclers were programmed to ramp between temperatures as rapidly as possible, yet ramp times did show variation. For instance, the time

required to ramp from 50° C to 72° C varied from 30 to 45 seconds. Similar variation due to thermocyclers would be expected between laboratories. As expected, primer sets varied for amplification and repeatability (Table 10).

Table 10. Number of primer sets that gave repeatable major and minor bands for four genotypes amplified by PCR relative to the total number of primer sets which amplified products.

Genotype	Number of repeatable primer sets/total number primers sets[a]					
	Major Bands			Minor Bands		
	Primers within genera[b]	Primers outside of genera[c]	Total[d]	Primers within genera[b]	Primers outside of genera[c]	Total[d]
Chinese Spring	23/42	24/44	47/86	5/42	5/45	10/87
Hi-Line	11/36	11/39	22/75	3/38	1/40	4/78
Chinook	28/44	6/33	34/77	0/35	2/36	2/71
Steptoe	31/44	13/36	44/80	5/37	1/36	6/73

[a] A primer set was defined as repeatable within a genotype if all major(or minor) bands amplified in all thermocyclers.
[b] Primers within genera: Primer sets derived from wheat or *T. tauschii* sequence used to amplify wheat; or primer sets derived from barley sequence used to amplify barley.
[c] Primers outside of genera: Primer sets developed from barley or oat sequence used to amplify wheat, or primers sets derived from wheat or oats sequence used to amplify barley.
[d] Total number of primer sets was less than 96 as some primer sets failed to amplify products in particular genotypes.

There were 318 measures of repeatability of major bands recorded, rather than the expected 384 (96 primer sets times four genotypes), as not all primer sets amplified products in all genotypes. Over all genotypes, approximately 50 % (147/318) of the primer sets

amplified all of their major bands across all four thermocyclers. Only 7 % of minor banding patterns were repeatable across thermocyclers.

Primer sets derived from cDNA and genomic clones, respectively, were equally repeatable for all four genotypes (data not shown). Primer sets developed from clones obtained from outside the target species influenced repeatability for the barley genotypes, Chinook and Steptoe, but not the wheat genotypes (Table 10). That is, amplification of wheat DNA with primer sets derived from barley or oats were equally repeatable as when wheat or *T. tauschii*-derived primer sets were used. *T. tauschii* is considered to be the same species as wheat for these experiments, in that sequence variation between the wheat D genome and *T. tauschii* is very low (Talbert et al., 1998; Blake et al., 1999). Repeatability was significantly higher when using barley-derived primer sets to amplify Chinook or Steptoe barley (χ^2, $P < .05$) as compared to using wheat or *T. tauschii*-derived primer sets.

Primer sets differed significantly for the number of major bands produced with up to 16 bands amplified for one primer/genotype combination. Primer sets which produced few bands were more repeatable with Steptoe barley ($P < .05$) based on an F test. This was not a significant factor for the other three genotypes.

Primer sets differed for all inherent molecular properties as described in Table 11. Several primer characteristics were identified as influencing repeatability of major bands (Table 12). GC content of primers was significantly related to repeatability of major bands for all four genotypes. Primers with high GC content resulted in lower repeatability. T_m-

Table 11. Means and standard deviations of the characteristics scored for the 96 primers used in this study.

Characteristic	Description	Mean and Standard Deviation Determined for each Characteristic[a]		
		Left Primer	Right Primer	Primer Pair
GC Content	% GC bases/total bases	50.1 (9.4)	48.5 (9.9)	NA
Primer-dimer Formation	composite score based on number of base matches and degree of 3' overhang	6.0 (6.5)	5.7 (6.0)	7.8 (5.6)
Hairpin Loop Stability	ΔG value (kcal/mol) for most stable hairpin structure determined by OLIGO program	1.9 (1.5)	1.2 (1.8)	NA
3'-end Stability	ΔG value (kcal/mol) of the 3'-terminal pentameter determined by OLIGO program	-7.4 (1.3)	-7.5 (1.3)	NA
T_m difference	difference between melting temperature of left and right primers (°C)	NA	NA	4.9 (4.3)
T_m-T_{ann}	difference between the primer with the highest melting temperature in a pair and the annealing temperature (°C)	NA	NA	15.2 (5.1)

[a] Mean is given first and standard deviation is listed second in parentheses.

T_{ann} had a significant impact on repeatability for Chinese Spring, Hi-Line and Steptoe. These results may be related to larger differences between annealing temperature and the T_m of the primers for primers with higher GC content. This difference could be minimized

Table 12. Characteristics of primer sets influencing repeatability of major bands for four genotypes. Characteristics significantly associated with repeatability are presented (P > .10).

Characteristic	Genotype	Repeatable primer sets		Non-repeatable primer sets		P value
		Mean	SD	Mean	SD	
Bands (number)	Steptoe	3.4	2.8	4.8	2.8	.029
Left GC[a] (%)	Chinese Spring	48.0	9.25	53.4	8.95	.007
	Hi-Line	46.8	8.7	50.9	9.5	.083
	Steptoe	47.6	9.2	54.2	9.1	.002
Right GC[b] (%)	Chinook	45.6	10.4	50.9	8.5	.017
	Steptoe	46.5	9.6	51.2	10.2	.038
HairpinL[a] (kcal/mol)	Chinook	2.7	1.8	1.5	1.0	.001
3'-end StabilityL[a]	Chinook	-7.0	1.3	-7.7	1.4	.018
	Steptoe	-7.0	1.3	-7.9	1.4	.005
3'-end StabilityR[b]	Hi-Line	-7.03	.95	-7.5	1.1	.103
	Chinook	-7.1	0.9	-7.6	1.2	.033
	Steptoe	-7.2	1.1	-7.7	1.3	.048
T_m-T_{ann} (°C)	Chinese Spring	14.6	5.0	16.6	5.2	.071
	Hi-Line	13.5	5.0	15.7	5.1	.081
	Steptoe	13.6	5.0	17.3	5.0	.001

[a] 'L' or 'Left'refers to the left primer. [b] 'R' or 'Right' refers to the right primer

by raising the annealing temperature during PCR. To determine whether higher annealing temperatures would increase repeatability for primers with high GC content and high T_m- T_{ann}, we selected twelve primers with high T_m- T_{ann} found to be non-repeatable at 50° C annealing temperature. The PCR reactions were repeated at 55 °C, along with a control reaction at 50 °C. We found that repeatability of major bands was improved for 4 of 12 primers for Steptoe, Chinook and Chinese Spring. Hi-Line showed improvement for only2 of 12 primers. No improvement was seen in repeatability of minor bands for any primers across all genotypes. We also observed that approximately one-quarter to one-third of the primer sets did not amplify when the annealing temperature was raised to 55 °C, depending on the genotype. The majority of primers (10/12) amplified fewer major and minor bands at the higher annealing temperature across all genotypes.

Stability of 3'-ends of the primers was also associated with repeatability for two genotypes where lower stability (higher ΔG value) resulted in more repeatable major bands. This is as expected, in that lower stability has been shown to decrease incidence of false priming (Rychlik, 1995).

No other characteristic of the primer sets influenced repeatability for more than one genotype. Many researchers have discussed the negative impact that primer-dimer formation has on PCR amplification (Innis and Gelfand, 1990, Dieffenbach et al., 1993, Brownie et al., 1997), but in our study this characteristic did not affect repeatability. Similarly, hairpin loop formation did not significantly impact repeatability in most genotypes. We also observed no significant effect of melting temperature difference between left and right primers.

Hi-Line wheat tended to be the least repeatable genotype, in that only 22/75 (29 %) of the primer sets gave a repeatable set of major bands. We suspected this may have been a property of the DNA preparation rather than the genotype. To test this supposition, we extracted genomic DNA from four plants each of Hi-Line and Chinese Spring. Four primer sets were selected which had initially been repeatable for major bands with Chinese Spring and not repeatable with Hi-Line. Re-amplification of the new DNA with these primer sets on all four thermocyclers showed that all major bands were repeatable for both genotypes (data not shown). This result suggests that minor differences between labs in the quality of DNA preparation may also influence repeatability. The fact that the quality of DNA preparation influences some primer sets more than others has previously been observed (Talbert et al., 1996).

The sharing of primer sequences between labs is likely to accelerate with genomics approaches, such as EST sequencing, providing an increased number of potentially useful marker loci. Past results with STS primer sets suggest that repeatability will be an important consideration, although little empirical data addressing primer characteristics that influence repeatability is available. Results of this study, and a previous one (Erpelding et al., 1996), suggest that primer sets derived from the non-target cereal species may not be as repeatable as those developed from within the target species. Additionally, high GC content and high internal stability of the 3'-end of the primers should be avoided to enhance repeatability. These factors should be considered in future primer set development.

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APPENDICES

APPENDIX A

PLANT MATERIAL

Nomenclature and Locations of Wild Wheat Species in this study

Lab designation	Accession	Species	Location
U1	Clae66	*Ae. umbellulata*	Yugoslavia,Serbia
U2	PI 204546	*Ae. umbellulata*	Turkey, Ankara
U3	PI 222762	*Ae. umbellulata*	Iran, Markazi
U4	PI 227339	*Ae. umbellulata*	Iran, Fars
U5	PI 227436	*Ae. umbellulata*	Iran, Fars
U6	PI 298906	*Ae. umbellulata*	Iraq
U7	PI 298907	*Ae. umbellulata*	Iraq
U8	PI 428569	*Ae. umbellulata*	Azerbaijan
U9	PI 486256	*Ae. umbellulata*	Turkey, Diyarbakir
U10	PI 487247	*Ae. umbellulata*	syria
U11	PI 542370	*Ae. umbellulata*	Turkey, Izmir
U12	PI 554405	*Ae. umbellulata*	Turkey, Denizli
U13	PI 573420	*Ae. umbellulata*	Turkey, Canakkale
U14	PI 573515	*Ae. umbellulata*	Turkey, Balikesir
U15	PI 573516	*Ae. umbellulata*	Turkey, Bilecik
U16	G631	*Ae. umbellulata*	Iran,Fars
U18	PI 542365	*Ae. umbellulata*	Turkey, Urfa
U19	PI 542368	*Ae. umbellulata*	Turkey, Maras
U20	PI 542369	*Ae. umbellulata*	Turkey, Usak
U21	PI 542379	*Ae. umbellulata*	Turkey, Izmir
U22	PI 542380	*Ae. umbellulata*	Turkey, Balikesir
U23	PI 542381	*Ae. umbellulata*	Turkey, Gaziantep
U24	PI 542382	*Ae. umbellulata*	Turkey, Gaziantep
U25	PI 542383	*Ae. umbellulata*	Turkey, Adiyaman
U26	PI 542384	*Ae. umbellulata*	Turkey, Adiyaman
U27	PI 554282	*Ae. umbellulata*	Turkey, Kocaeli
U28	PI 554385	*Ae. umbellulata*	Turkey, Kirsehir
U29	PI 554411	*Ae. umbellulata*	Turkey, Konya
U30	PI 554412	*Ae. umbellulata*	Turkey, Kirsehir
U31	PI 554413	*Ae. umbellulata*	Turkey, Malatya
U32	PI 554415	*Ae. umbellulata*	Turkey, Elazig
U33	PI 554416	*Ae. umbellulata*	Turkey, Hakkari
U34	PI 554417	*Ae. umbellulata*	Turkey, Corum
U35	PI 560555	*Ae. umbellulata*	Turkey, Siirt

Total of *Ae. umbellulata* 34 accessions

Lab designation	Accession	Species	Location
C1	PI 551142	*Ae. caudata*	Greece, Peloponnese
C2	PI 560732	*Ae. caudata*	Turkey, Bitlis
C3	PI 564195	*Ae. caudata*	Turkey, Canakkale
C4	PI 573414	*Ae. caudata*	Turkey, Eskisekir
C5	PI 573416	*Ae. caudata*	Turkey, Bilecik
C7	PI 551129	*Ae. caudata*	Greece, Central Greece
C8	PI 551128	*Ae. caudata*	Greece, Central Greece
C9	PI 551123	*Ae. caudata*	Greece, Macedonia
C10	PI 551121	*Ae. caudata*	Greece, Thessaly
C11	PI 542203	*Ae. caudata*	Turkey, Denizli
C12	PI 542202	*Ae. caudata*	Turkey, Izmir
C13	PI 5422198	*Ae. caudata*	Turkey, Diyarbakir
C14	PI 542199	*Ae. caudata*	Turkey, Urfa
C15	PI 5422201	*Ae. caudata*	Turkey, Gaziantep
C16	PI 542200	*Ae. caudata*	Turkey, Urfa
C17	TA # 2086	*Ae. caudata*	Turkey, Balikesir
C18	TA # 2096	*Ae. caudata*	Turkey, Antalya
C19	PI 542205	*Ae. caudata*	Turkey, Denizli
C20	PI 542206	*Ae. caudata*	Turkey, Denizli
C21	PI 542207	*Ae. caudata*	Turkey, Izmir
C22	PI 542208	*Ae. caudata*	Turkey, Balikesir
C23	PI 542209	*Ae. caudata*	Turkey, Gaziantep
C24	PI 542219	*Ae. caudata*	Turkey, Izmir
C25	PI 551130	*Ae. caudata*	Greece, Central Greece
C26	PI 551146	*Ae. caudata*	Greece, Peloponnese
C27	PI 551148	*Ae. caudata*	Greece, Peloponnese
C28	PI 554194	*Ae. caudata*	Turkey, Denizli
C29	PI 560731	*Ae. caudata*	Turkey, Siirt
C30	PI 564197	*Ae. caudata*	Turkey, Canakkale
C31	PI 573412	*Ae. caudata*	Turkey, Balikesir
C32	PI 573413	*Ae. caudata*	Turkey, Eskisekir
C33	PI 573417	*Ae. caudata*	Turkey, Bilecik
C34	PI 573418	*Ae. caudata*	Turkey, Cankiri

Total of *Ae. caudata* 33 accessions

Lab designation	Accession	Species	Location
UC1	PI 542322	*Ae. triuncialis ssp. triuncialis*	Turkey, Denizli
UC2	PI 542279	*Ae. triuncialis ssp. triuncialis*	Turkey, Diyarbakir
UC3	PI 487201	*Ae. triuncialis ssp. triuncialis*	Syria
UC4	PI 374344	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Serbia
UC5	PI 226501	*Ae. triuncialis ssp. triuncialis*	Iran, Fars
UC6	PI 219864	*Ae. triuncialis ssp. triuncialis*	Iraq
UC7	PI 542325	*Ae. triuncialis ssp. triuncialis*	Turkey, Denizli
UC8	PI 542335	*Ae. triuncialis ssp. triuncialis*	Turkey, Izmir
UC9	PI 542336	*Ae. triuncialis ssp. triuncialis*	Turkey, Balikesir
UC10	PI 542339	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC11	PI 551178	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC12	PI 551195	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC13	PI 551224	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC14	PI 573462	*Ae. triuncialis ssp. triuncialis*	Turkey, Bilecik
UC15	PI 574471	*Ae. triuncialis ssp. triuncialis*	Azerbaijan
UC18	PI 573500	*Ae. triuncialis ssp. triuncialis*	Turkey, Cankiri
UC19	PI 220330	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Faryab
UC20	PI 220332	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Heart
UC21	TA # 2229	*Ae. triuncialis ssp. triuncialis*	Morocco, Fes
UC22	PI 170192	*Ae. triuncialis ssp. triuncialis*	Turkey, Izmir
UC23	PI 170197	*Ae. triuncialis ssp. triuncialis*	Turkey, Kirklareli
UC24	PI 170201	*Ae. triuncialis ssp. triuncialis*	Turkey, Edirne
UC25	PI 170202	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC26	PI 170207	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC27	PI 170211	*Ae. triuncialis ssp. triuncialis*	Turkey, Balikesir
UC28	PI 170212	*Ae. triuncialis ssp. triuncialis*	Turkey, Balikesir
UC29	PI 171468	*Ae. triuncialis ssp. triuncialis*	Turkey, Tokat
UC30	PI 172682	*Ae. triuncialis ssp. triuncialis*	Turkey, Kars
UC31	PI 172684	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC32	PI 173615	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC33	PI 178820	*Ae. triuncialis ssp. triuncialis*	Turkey, Kirklareli
UC34	PI 180793	*Ae. triuncialis ssp. triuncialis*	Turkey, Istanbul
UC35	PI 203436	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC36	PI 203437	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC37	PI 204853	*Ae. triuncialis ssp. triuncialis*	Turkey, Elazig
UC38	PI 215781	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Badakhshan
UC39	PI 220327	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Baghlan
UC40	PI 223321	*Ae. triuncialis ssp. triuncialis*	Iran, East Azerbaijan

Lab designation	Accession	Species	Location
UC41	PI 227291	*Ae. triuncialis ssp. triuncialis*	Iran, Khuzestan
UC42	PI 227292	*Ae. triuncialis ssp. triuncialis*	Iran, Khuzestan
UC43	PI 227437	*Ae. triuncialis ssp. triuncialis*	Iran, Fars
UC44	PI 250696	*Ae. triuncialis ssp. triuncialis*	Iran, East Azerbaijan
UC45	PI 250908	*Ae. triuncialis ssp. triuncialis*	Iran, East Azerbaijan
UC46	PI 254860	*Ae. triuncialis ssp. triuncialis*	Iraq
UC47	PI 254861	*Ae. triuncialis ssp. triuncialis*	Iraq
UC48	PI 268206	*Ae. triuncialis ssp. triuncialis*	Iran, Ilam
UC49	PI 276990	*Ae. triuncialis ssp. triuncialis*	Turkey
UC50	PI 276991	*Ae. triuncialis ssp. triuncialis*	Afghanistan
UC51	PI 276992	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Baghlan
UC52	PI 317391	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Heart
UC53	PI 317399	*Ae. triuncialis ssp. triuncialis*	Afghanistan, Samangan
UC54	PI 344783	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC55	PI 344792	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC56	PI 344793	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC57	PI 344795	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC58	PI 344796	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC59	PI 349039	*Ae. triuncialis ssp. triuncialis*	Georgia
UC60	PI 361882	*Ae. triuncialis ssp. triuncialis*	Romania, Cluj
UC61	PI 374327	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC62	PI 374331	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC63	PI 374340	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC64	PI 374342	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC65	PI 374343	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC66	PI 374346	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC67	PI 374349	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Serbia
UC68	PI 374350	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Serbia
UC69	PI 374351	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC70	PI 374354	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC71	PI 374356	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC72	PI 374357	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Serbia
UC73	PI 374359	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC74	PI 374362	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC75	PI 374363	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC76	PI 374366	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Montenegro
UC77	PI 374372	*Ae. triuncialis ssp. triuncialis*	Macedonia
UC78	PI 374376	*Ae. triuncialis ssp. triuncialis*	Macedonia

Lab designation	Accession	Species	Location
UC79	PI 374379	*Ae. triuncialis ssp. triuncialis*	Yugoslavia, Serbia
UC80	PI 383529	*Ae. triuncialis ssp. triuncialis*	Turkey, Gaziantep
UC81	PI 407638	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC82	PI 428559	*Ae. triuncialis ssp. triuncialis*	Azerbaijan
UC83	PI 428567	*Ae. triuncialis ssp. triuncialis*	Azerbaijan
UC84	PI 428568	*Ae. triuncialis ssp. triuncialis*	Azerbaijan
UC85	PI 483037	*Ae. triuncialis ssp. triuncialis*	Cyprus
UC86	PI 486279	*Ae. triuncialis ssp. triuncialis*	Turkey, Kayseri
UC87	PI 486280	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC88	PI 486283	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC89	PI 486285	*Ae. triuncialis ssp. triuncialis*	Turkey, Kayseri
UC90	PI 486286	*Ae. triuncialis ssp. triuncialis*	Turkey, Malatya
UC91	PI 486287	*Ae. triuncialis ssp. triuncialis*	Turkey, Malatya
UC92	PI 486288	*Ae. triuncialis ssp. triuncialis*	Turkey, Elazig
UC93	PI 486289	*Ae. triuncialis ssp. triuncialis*	Turkey, Elazig
UC94	PI 486290	*Ae. triuncialis ssp. triuncialis*	Turkey, Elazig
UC95	PI 486291	*Ae. triuncialis ssp. triuncialis*	Turkey, Diyarbakir
UC96	PI 486292	*Ae. triuncialis ssp. triuncialis*	Turkey, Diyarbakir
UC97	PI 486294	*Ae. triuncialis ssp. triuncialis*	Turkey, Bitlis
UC98	PI 486295	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC99	PI 486296	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC100	PI 486298	*Ae. triuncialis ssp. triuncialis*	Turkey, Agri
UC101	PI 486299	*Ae. triuncialis ssp. triuncialis*	Turkey, Kars
UC102	PI 486301	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC103	PI 487239	*Ae. triuncialis ssp. triuncialis*	Syria
UC104	PI 487240	*Ae. triuncialis ssp. triuncialis*	Syria
UC105	PI 487241	*Ae. triuncialis ssp. triuncialis*	Syria
UC106	PI 487242	*Ae. triuncialis ssp. triuncialis*	Syria
UC107	PI 487243	*Ae. triuncialis ssp. triuncialis*	Syria
UC108	PI 487244	*Ae. triuncialis ssp. triuncialis*	Syria
UC109	PI 487245	*Ae. triuncialis ssp. triuncialis*	Syria
UC110	PI 487246	*Ae. triuncialis ssp. triuncialis*	Syria
UC111	PI 491438	*Ae. triuncialis ssp. triuncialis*	France, Var
UC112	PI 491442	*Ae. triuncialis ssp. triuncialis*	France, Var
UC113	PI 524957	*Ae. triuncialis ssp. triuncialis*	Italy, Sicily
UC114	PI 542301	*Ae. triuncialis ssp. triuncialis*	Turkey, Urfa
UC115	PI 542302	*Ae. triuncialis ssp. triuncialis*	Turkey, Urfa
UC116	PI 542303	*Ae. triuncialis ssp. triuncialis*	Turkey, Adiyaman

Lab designation	Accession	Species	Location
UC117	PI 542304	*Ae. triuncialis ssp. triuncialis*	Turkey, Adiyaman
UC118	PI 542305	*Ae. triuncialis ssp. triuncialis*	Turkey, Maras
UC119	PI 542306	*Ae. triuncialis ssp. triuncialis*	Turkey, Maras
UC120	PI 542307	*Ae. triuncialis ssp. triuncialis*	Turkey, Gaziantep
UC121	PI 542308	*Ae. triuncialis ssp. triuncialis*	Turkey, Gaziantep
UC122	PI 542309	*Ae. triuncialis ssp. triuncialis*	Turkey, Konya
UC123	PI 542310	*Ae. triuncialis ssp. triuncialis*	Turkey, Usak
UC124	PI 542311	*Ae. triuncialis ssp. triuncialis*	Turkey, Usak
UC125	PI 542312	*Ae. triuncialis ssp. triuncialis*	Turkey, Manisa
UC126	PI 542313	*Ae. triuncialis ssp. triuncialis*	Turkey, Manisa
UC127	PI 542314	*Ae. triuncialis ssp. triuncialis*	Turkey, Izmir
UC128	PI 542315	*Ae. triuncialis ssp. triuncialis*	Turkey, Izmir
UC129	PI 551179	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC130	PI 551180	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC131	PI 551181	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC132	PI 551182	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC133	PI 551183	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC134	PI 551185	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC135	PI 551186	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC136	PI 551187	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC137	PI 551188	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC138	PI 551189	*Ae. triuncialis ssp. triuncialis*	Greece, Thessaly
UC139	PI 551201	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC140	PI 551202	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC141	PI 551203	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC142	PI 551204	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC143	PI 551205	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC144	PI 551206	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC145	PI 551207	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC146	PI 551208	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC147	PI 551209	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC148	PI 551210	*Ae. triuncialis ssp. triuncialis*	Greece, Macedonia
UC149	PI 551211	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC150	PI 551212	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC151	PI 551213	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC152	PI 551214	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC153	PI 551215	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC154	PI 551216	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece

Lab designation	Accession	Species	Location
UC155	PI 551217	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC156	PI 551218	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC157	PI 551219	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC158	PI 551220	*Ae. triuncialis ssp. triuncialis*	Greece, Central Greece
UC159	PI 551227	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC160	PI 551228	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC161	PI 551229	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC162	PI 551230	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC163	PI 551231	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC164	PI 551232	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC165	PI 551233	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC166	PI 551234	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC167	PI 551235	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC168	PI 551236	*Ae. triuncialis ssp. triuncialis*	Greece, Peloponnese
UC169	PI 554351	*Ae. triuncialis ssp. triuncialis*	Turkey, Diyarbakir
UC170	PI 554352	*Ae. triuncialis ssp. triuncialis*	Turkey, Diyarbakir
UC171	PI 554355	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC172	PI 554356	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC173	PI 554358	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC174	PI 554381	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC175	PI 554382	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC176	PI 554383	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC177	PI 560539	*Ae. triuncialis ssp. triuncialis*	Turkey, Van
UC178	PI 560540	*Ae. triuncialis ssp. triuncialis*	Turkey, Siirt
UC179	PI 560541	*Ae. triuncialis ssp. triuncialis*	Turkey, Bitlis
UC180	PI 560542	*Ae. triuncialis ssp. triuncialis*	Turkey, Siirt
UC181	PI 560543	*Ae. triuncialis ssp. triuncialis*	Turkey, Siirt
UC182	PI 560545	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC183	PI 560547	*Ae. triuncialis ssp. triuncialis*	Turkey, Hakkari
UC184	PI 560548	*Ae. triuncialis ssp. triuncialis*	Turkey, Bitlis
UC185	PI 560549	*Ae. triuncialis ssp. triuncialis*	Turkey, Bitlis
UC186	PI 560550	*Ae. triuncialis ssp. triuncialis*	Turkey, Bitlis
UC187	PI 564225	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC188	PI 564226	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC189	PI 564227	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC190	PI 564228	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC191	PI 564230	*Ae. triuncialis ssp. triuncialis*	Turkey, Balikesir
UC192	PI 564231	*Ae. triuncialis ssp. triuncialis*	Turkey, Balikesir

Lab designation	Accession	Species	Location
UC193	PI 564233	*Ae. triuncialis ssp. triuncialis*	Turkey, Bursa
UC194	PI 568163	*Ae. triuncialis ssp. triuncialis*	Uzbekistan
UC195	PI 573451	*Ae. triuncialis ssp. triuncialis*	Turkey, Ankara
UC196	PI 573453	*Ae. triuncialis ssp. triuncialis*	Turkey, Canakkale
UC197	PI 573454	*Ae. triuncialis ssp. triuncialis*	Turkey, Bursa
UC198	PI 574470	*Ae. triuncialis ssp. triuncialis*	Uzbekistan
UC199	PI 574472	*Ae. triuncialis ssp. triuncialis*	Azerbaijan
UC200	PI 574473	*Ae. triuncialis ssp. triuncialis*	Turkmenistan
UC201	PI 574474	*Ae. triuncialis ssp. triuncialis*	Bulgaria
UC202	PI 614630	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
UC203	PI 614631	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
UC204	PI 614632	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
UC205	PI 614633	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
UC206	PI 614634	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
UC207	PI 614635	*Ae. triuncialis ssp. triuncialis*	Ukraine, Krym
KU2505		*Ae. triuncialis ssp. triuncialis*	Afghanistan
KU2517		*Ae. triuncialis ssp. triuncialis*	Afghanistan
KU6904		*Ae. triuncialis ssp. triuncialis*	Greece
KU6909			Cyprus

Total of *Ae. triuncialis ssp. triuncialis* 209accessions

Lab designation	Accession	Species	Location
UC P2	PI 219866	*Ae. triuncialis ssp. persica*	Iraq
UC P3	PI 220328	*Ae. triuncialis ssp. persica*	Afghanistan, Kondoz
UC P4	PI 220329	*Ae. triuncialis ssp. persica*	Afghanistan, Faryab
UC P5	PI 227340	*Ae. triuncialis ssp. persica*	Iran, Fars
UC P6	PI 317395	*Ae. triuncialis ssp. persica*	Afghanistan, Heart
UC P7	PI 317396	*Ae. triuncialis ssp. persica*	Afghanistan, Kondoz
UC P8	PI 317397	*Ae. triuncialis ssp. persica*	Afghanistan, Kondoz
UC P9	PI 483027	*Ae. triuncialis ssp. persica*	Cyprus
UC P10	PI 483029	*Ae. triuncialis ssp. persica*	Cyprus
UC P11	CIae 69	*Ae. triuncialis ssp. persica*	unknown

Total of *Ae. triuncialis ssp. persica*
11accessions

Lab designation	Accession	Species	Location
TA2427	KU2050	*Triticum tauschii*	Afghanistan

Outgroup 1 accession

APPENDIX B.

BANDING PATTERN RESULTS

Lab designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
U01	A	L(T)	3	2	1	2
U02	A	L(T)	1	1	1	1
U03	B	L(T)	1	1	1	1
U04	B	L(T)	1	1	1	1
U05	A	L(T)	1	1	1	1
U06	B	L	1	1	1	1
U07	B	L(T)	1	1	1	1
U08	B	L(T)	4	1	1	1
U09	B	L(T)	1	1	NA	1
U10	A	L(T)	5	10	2	1
U11	A	L	1	1	1	1
U12	B	L	1	1	1	1
U13	A	L	1	3	1	1
U14	B	L(T)	1	1	1	1
U15	B	L	1	1	1	1
U16	B	L	1	1	1	1
U18	NA	D	1	1	1	NA
U19	NA	L	1	4	NA	NA
U20	A	L	1	1	1	1
U21	B	D	1	1	1	1
U22	B	L	1	1	1	1
U23	NA	L	1	1	1	1
U24	NA	NA	1	5	1	1
U25	NA	L	1	6	1	1
U26	B	D	1	1	1	1
U27	A	L	1	2	1	3
U28	B	L	1	1	1	1
U29	B	L	1	1	1	1
U30	B	L	2	1	NA	NA
U31	B	L	1	1	1	1
U32	NA	L	1	1	1	1
U33	B	L	1	1	1	1
U34	A	L	2	1	NA	NA
U35	A	L	1	5	1	1

G43 Locus: Allele A and B; NA = No amplification.
U6/R6 Locus: D = deletion; L = no deletion; NA = No amplification
L(T) = no deletion with nucleotide T at position 58329.
Residual loci: Pattern number (1,2,3.....); NA = No amplification

Lab designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
C01	NA	L(G)	1	1	1	1
C02	NA	D	5	10	NA	NA
C03	NA	D	5	NA	3	NA
C04	NA	D	5	NA	3	NA
C05	NA	D	5	NA	3	NA
C07	NA	D	5	7	3	NA
C08	NA	L(G)	5	5	3	NA
C09	NA	D	5	NA	3	4
C10	NA	D	6	8	3	4
C11	NA	D	5	NA	3	NA
C12	NA	D	5	NA	3	4
C13	NA	D	5	NA	3	NA
C14	NA	D	5	NA	3	NA
C15	NA	D	5	NA	3	NA
C16	NA	D	6	NA	3	NA
C17	NA	L(G)	7	9	NA	NA
C18	NA	D	7	NA	NA	NA
C19	NA	NA	NA	NA	NA	NA
C20	NA	NA	8	NA	3	1
C21	NA	D	7	NA	3	NA
C22	NA	D	7	NA	3	5
C23	NA	D	5	NA	3	5
C24	NA	D	6	NA	3	NA
C25	NA	NA	NA	NA	NA	NA
C26	NA	D	NA	NA	NA	NA
C27	NA	L(G)	NA	NA	NA	NA
C28	NA	NA	9	1	2	5
C29	NA	D	6	NA	3	NA
C30	NA	D	6	NA	5	NA
C31	NA	D	6	NA	NA	5
C32	NA	NA	5	1	4	5
C33	NA	NA	5	NA	5	5
C34	NA	D	6	NA	NA	5

G43 Locus: Allele A and B; NA = No amplification.
U6/R6 Locus: D = deletion; L = no deletion; NA = No amplification
L(G) = no deletion with nucleotide G at position 58329.
Residual loci: Pattern number (1,2,3.....); NA = No amplification

Lab designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
UC01	B	D	4	1	NA	1
UC02	A	L(T)	5	1	NA	1
UC03	B	L(G)	5	1	NA	1
UC04	B	D	5	1	NA	1
UC05	B	L(T)	11	1	NA	1
UC06	B	D	5	1	NA	1
UC07	B	D	4	1	NA	1
UC08	B	L(T)	NA	1	NA	1
UC09	A	L	5	1	NA	1
UC10	A	L	4	1	NA	1
UC11	A	L(T)	5	1	NA	5
UC12	A	L(G)	5	1	NA	1
UC13	A	L(T)	4	1	NA	5
UC14	A	D	5	1	NA	1
UC15	B	D	5	1	NA	1
UC18	B	D	NA	1	NA	1
UC19	B	D	NA	1	NA	1
UC20	B	L	5	1	NA	5
UC21	B	D	5	1	NA	1
UC22	NA	D	NA	NA	NA	1
UC23	B	L	4	1	6	NA
UC24	B	D	NA	NA	NA	NA
UC25	NA	L	NA	1	NA	1
UC26	B	L	NA	1	NA	1
UC27	A	L(T)	NA	NA	NA	1
UC28	B	L	NA	1	NA	1
UC29	B	L	NA	1	NA	1
UC30	A	D	4	1	6	1
UC31	B	L	12	9	1	7
UC32	A	D	5	1	6	1
UC33	A	L	4	NA	NA	NA
UC34	B	D	4	1	1	1
UC35	B	D	4	1	6	1
UC36	B	L	4	1	6	1
UC37	B	L	5	1	1	1
UC38	B	D	5	1	7	1
UC39	B	D	5	1	7	1
UC40	B	L(G)	4	1	7	1

Lab designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
UC41	B	L	10	1	1	1
UC42	B	L(G)	NA	NA	1	1
UC43	B	D	5	1	1	1
UC44	A	D	4	1	7	1
UC45	B	D	NA	1	1	1
UC46	A	D	NA	1	NA	1
UC47	A	D	NA	1	4	1
UC48	B	L(G)	NA	NA	NA	1
UC49	A	L	NA	1	NA	1
UC50	B	L(G)	5	1	6	1
UC51	B	D	5	1	NA	1
UC52	A	L	NA	1	NA	1
UC53	B	L	NA	1	NA	1
UC54	A	L	NA	1	NA	1
UC55	B	L	NA	1	6	8
UC56	B	D	NA	1	NA	1
UC57	A	L	NA	1	NA	8
UC58	B	D	4	1	7	1
UC59	B	D	5	1	4	1
UC60	B	D	NA	1	NA	1
UC61	B	D	NA	1	NA	1
UC62	B	D	5	1	NA	1
UC63	B	L	5	1	7	1
UC64	A	L	NA	1	NA	NA
UC65	B	L	5	1	7	8
UC66	B	D	5	1	NA	1
UC67	B	D	5	1	NA	1
UC68	A	D	5	1	NA	9
UC69	A	D	12	1	NA	9
UC70	B	L	5	1	7	1
UC71	B	L	5	1	7	1
UC72	B	L	5	NA	NA	NA
UC73	B	L	5	1	7	1
UC74	A	L	4	1	6	1
UC75	A	L	4	1	7	1
UC76	B	L	5	1	6	1
UC77	A	L	4	1	NA	1
UC78	B	L	5	1	NA	NA
UC79	A	D	4	1	6	1
UC80	B	D	NA	1	NA	1

Lab designation	G4B3 /*DdBe*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
UC81	B	D	NA	NA	NA	1
UC82	B	L	12	2	1	7
UC83	B	D	NA	1	4	1
UC84	B	D	5	1	11	1
UC85	B	L	5	1	2	1
UC86	B	L	NA	1	11	1
UC87	B	D	5	1	6	1
UC88	A	L	5	1	7	1
UC89	A	L	NA	1	6	1
UC90	B	D	NA	NA	NA	1
UC91	B	D	5	1	6	1
UC92	A	D	4	1	6	1
UC93	A	D	NA	1	NA	1
UC94	B	L	NA	NA	NA	1
UC95	B	L	NA	NA	NA	1
UC96	A	D	NA	1	4	1
UC97	B	NA	NA	1	NA	1
UC98	B	D	NA	1	NA	1
UC99	B	D	NA	NA	NA	1
UC100	B	D	4	1	2	1
UC101	B	D	4	1	2	1
UC102	A	D	NA	NA	NA	1
UC103	NA	D	NA	1	NA	1
UC104	A	L	NA	NA	NA	1
UC105	B	L	NA	NA	4	1
UC106	NA	D	NA	NA	NA	1
UC107	B	D	NA	NA	NA	1
UC108	B	D	NA	NA	NA	NA
UC109	A	D	NA	NA	NA	1
UC110	NA	D	NA	NA	NA	NA
UC111	A	D	NA	NA	NA	1
UC112	B	D	NA	NA	NA	1
UC113	NA	D	NA	NA	NA	NA
UC114	B	L	NA	NA	NA	1
UC115	B	D	5	1	8	1
UC116	A	L	5	1	13	1
UC117	B	L	NA	1	14	1
UC118	NA	NA	NA	NA	NA	NA
UC119	NA	D	NA	NA	NA	NA
UC120	A	D	NA	NA	NA	NA

Lab Designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
UC121	NA	D	NA	NA	9	NA
UC122	B	D	NA	1	9	1
UC123	B	D	NA	7	NA	5
UC124	A	D	NA	NA	NA	NA
UC125	B	D	NA	NA	10	1
UC126	A	L	NA	NA	NA	1
UC127	A	L	NA	1	9	1
UC128	A	L	NA	NA	9	1
UC129	A	L	4	1	9	9
UC130	A	L	4	1	9	1
UC131	NA	S	NA	NA	NA	NA
UC132	A	L	NA	NA	NA	1
UC133	A	L	NA	NA	NA	NA
UC134	A	L	NA	NA	NA	9
UC135	NA	D	NA	NA	NA	NA
UC136	A	D	NA	NA	NA	5
UC137	A	L	5	1	9	NA
UC138	A	D	NA	NA	NA	NA
UC139	A	L	NA	1	5	NA
UC140	B	L	4	1	9	NA
UC141	A	L	NA	1	9	NA
UC142	A	D	NA	NA	NA	NA
UC143	NA	NA	NA	NA	NA	NA
UC144	A	L	NA	NA	NA	1
UC145	A	L	NA	NA	NA	1
UC146	A	L	4	1	11	1
UC147	A	L	NA	1	10	6
UC148	A	L	5	1	10	1
UC149	B	NA	NA	NA	NA	NA
UC150	B	L	NA	NA	NA	1
UC151	B	L	5	1	7	1
UC152	B	L	4	1	7	1
UC153	B	L	5	1	7	1
UC154	A	L	5	1	6	1
UC155	B	L	4	1	6	1
UC156	B	L	NA	1	7	1
UC157	B	L	4	1	NA	1
UC158	A	L	NA	1	7	1
UC159	B	L	NA	NA	12	1
UC160	A	L	NA	1	7	6

Lab Designation	G43 /*Dde*I	U6/R6	D2 /*Rsa*I	D15 /*Hha*I	F8 /*Hinf*I	WG232 /*Mse*I
UC161	B	D	NA	1	NA	1
UC162	B	L	NA	NA	NA	1
UC163	A	D	NA	NA	NA	1
UC164	NA	D	12	1	NA	NA
UC165	NA	D	NA	NA	NA	NA
UC166	A	L	NA	NA	NA	1
UC167	NA	D	NA	1	NA	NA
UC168	NA	NA	NA	NA	NA	NA
UC169	NA	D	4	1	NA	NA
UC170	NA	NA	NA	NA	NA	NA
UC171	B	D	4	1	NA	1
UC172	B	D	4	1	NA	1
UC173	B	L	NA	NA	NA	1
UC174	NA	NA	12	1	NA	NA
UC175	B	D	NA	NA	NA	1
UC176	B	D	4	1	NA	1
UC177	B	L	NA	1	NA	1
UC178	NA	NA	12	1	NA	NA
UC179	B	L	NA	1	NA	1
UC180	NA	NA	NA	NA	NA	NA
UC181	B	D	NA	1	NA	1
UC182	A	L	NA	1	NA	1
UC183	B	D	12	1	NA	1
UC184	B	L	NA	1	NA	1
UC185	NA	D	12	1	NA	NA
UC186	B	D	NA	1	NA	1
UC187	A	L	4	1	NA	1
UC188	A	L	NA	1	NA	1
UC189	B	L	NA	1	NA	1
UC190	B	L	NA	1	NA	1
UC191	B	L	NA	1	NA	1
UC192	A	L	NA	1	NA	1
UC193	NA	L	12	1	NA	1
UC194	B	D	NA	1	NA	1
UC195	A	D	4	11	NA	1
UC196	B	L	4	1	NA	1
UC197	A	L	12	1	NA	1
UC198	B	D	4	1	NA	1
UC199	A	D	4	1	NA	1
UC200	A	L(G)	12	1	NA	1

Lab Designation	G43 */Dde*I	U6/R6	D2 */Rsa*I	D15 */Hha*I	F8 */Hinf*I	WG232 */Mse*I
UC201	B	D	4	1	NA	1
UC202	A	D	5	1	NA	1
UC203	B	L	NA	1	NA	1
UC204	B	D	NA	12	NA	1
UC205	B	L	10	1	NA	1
UC206	B	L	12	1	NA	NA
UC207	B	L	NA	NA	NA	NA
KU2505	B	D	12	1	NA	5
KU2517	B	D	12	1	NA	5
KU6904	A	L	NA	1	NA	5
KU6909	B	D	4	1	NA	5
TA2427	A'	L(G)	NA	9	NA	5
UC P01	NA	D				
UC P02	NA	NA				
UC P03	NA	D				
UC P04	NA	D				
UC P05	NA	NA				
UC P06	NA	NA				
UC P07	NA	NA				
UC P08	NA	NA				
UC P09	NA	NA				
UC P10	NA	D				
UC P11	B	D				

G43 Locus: Allele A, B and A'; NA = No amplification.

U6/R6 Locus: D = deletion; L = no deletion; NA = No amplification

L(T) = no deletion with nucleotide T at position 58329.

L(G) = no deletion with nucleotide G at the same position.

Residual loci: Pattern number (1,2,3.....); NA = No amplification

APPENDIX C

ALIGNMENT OF DNA SEQUENCES

G43 Locus

```
#nexus
[Subset data]
BEGIN DATA;
  DIMENSIONS NTAX=19  NCHAR= 802;
   FORMAT
    INTERLEAVE
      MISSING=? GAP=- MATCHCHAR=.
        DATATYPE=DNA
;
MATRIX
            [         1 1111111112 2222222223 3333333334 4444444445 5555555556
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    GGCACATCAT GCACACGATG TTAATCCTTT TTTTT-ATTT CC-CGTCAGA TGAGAATGGG
43U02.SEQ    .......... .......... .......... .....T.... ..A....... .......T..
43U03.SEQ    ......A... ......T... .......... .....T.... .......... .......T..
43U04.SEQ    ......A... ......T... .......... .....T.... ..A....... .......T..
43U05.SEQ    .......... .......... .......... .....T.... ..A....... .......T..
43U07.SEQ    ......A... ......T... .......... .....T.... ..A....... .......T..
43U08.SEQ    ......A... ......T... .......... .....T.... ..A....... .......T..
43U09.SEQ    ......A... ......T... .......... .......... ..A....... .......T..
43U10.SEQ    .......... .......... .......... .....T.... ..A....... .......T..
43U14.SEQ    ......A... ......T... .......... .....T.... ..A....... .......T..
43UC01.SEQ   ......A... ......T... .......... .....T.... ..A....... .......T..
43UC02.SEQ   .......... .......... .......... .....T.... ..A....... .......T..
43UC04.SEQ   ......A... ......T... .......... .......... ..A....... .......T..
43UC05.SEQ   ......A... ......T... .......... .......... ..A....... .......T..
43UC06.SEQ   ......A... ......T... .......... .......... ..A....... .......T..
43UC07.SEQ   ......A... ......T... .......... .......... ..A....... .......T..
43UC11.SEQ   .......... .......... .......... .....T.... ..A....... .......T..
43UC15.SEQ   ......A... ......T... .......... .......... ..A....... .......T..
43TA.SEQ     .......... .......... .......-.. .......... .......... .......TC.


            [                                                 1 1111111111 1111111111
             6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    TAAAACCACA AAATTAATCG AGACATGCTA AAACATCTGT ACTTAAAAAA --CATGCA--
43U02.SEQ    .......... .......... .......... .......... ....-..... ..........
43U03.SEQ    .......... .......... .......... .......... ....-.G... ..........
43U04.SEQ    .......... .......... .......... .......... ....-.G... ..........
43U05.SEQ    .......... .......... .......... .......... ....-..... ..........
43U07.SEQ    .......... .......... .......... .......... ....-.G... ..........
43U08.SEQ    .......... .......... .......... .......... ....-.G... ..........
43U09.SEQ    .......... .......... .......... .......... ....-.G... ..........
43U10.SEQ    .......... .......... .......... .......... ....-..... ..........
43U14.SEQ    .......... .......... .......... .......... ....-.G... ..........
43UC01.SEQ   .......... .......... .......... .......... ....-.G... ..........
43UC02.SEQ   .......... .......... .......... .......... ....-..... ..........
43UC04.SEQ   .......... .......... .......... .......... ....-.G... ..........
43UC05.SEQ   .......... .......... .......... .......... ....-.G... ..........
43UC06.SEQ   .......... .......... .......... .......... ....-.G... ..........
43UC07.SEQ   .......... .......... .......... .......... ....-.G... ..........
43UC11.SEQ   .......... .......... .......... .......... ....-..... ..........
43UC15.SEQ   .......... .......... .......... .......... ....-.G... ..........
43TA.SEQ     .......... .........C .......... ...A.....C .........C AC..A...GC
```

```
           [1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
            2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
            1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ   -TAGAGAAAC CTGTAAATAA AGATTAAAAA TACGGCTAGT GG-------- --------AC
43U02.SEQ   .......G.. ...----... .......... .......... .......... ..........
43U03.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43U04.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43U05.SEQ   .......G.. ...----... .......... .......... .......... ..........
43U07.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43U08.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43U09.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43U10.SEQ   .......G.. ...----... .......... .......... .......... ..........
43U14.SEQ   .......G.. ...----... ....A..... .......... .......... ..........
43UC01.SEQ  .......G.. ...----... ....A..... .......... .......... ..........
43UC02.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC04.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC05.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC06.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC07.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC11.SEQ  .......G.. ...----... .......... .......... .......... ..........
43UC15.SEQ  .......G.. ...----... .......... .......... .......... ..........
43TA.SEQ    C......... ...----..C .......... .......... ..CGTTTCAA AAAAATTA.A


           [1111111111 1111111112 2222222222 2222222222 2222222222 2222222222
            8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
            1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ   AAG--TAGT- -------AGG ACAGCACGAC AAATGTACAT GAGTACGCCA TTCTCCGCAT
43U02.SEQ   .......... .......... ......T... .C.......G ......A... ..........
43U03.SEQ   .......... .......... .......... .......... .......... ..........
43U04.SEQ   .......... .......... .......... .......... .......... ..........
43U05.SEQ   .......... .......... ......T... .C.......G ......A... ..........
43U07.SEQ   .......... .......... .......... .......... .......... ..........
43U08.SEQ   .......... .......... .......... .......... .......... ..........
43U09.SEQ   .......... .......... .......... .......... .......... ..........
43U10.SEQ   .......... .......... ......T... .C.......G ......A... ..........
43U14.SEQ   .......... .......... .......... .......... .......... ..........
43UC01.SEQ  .......... .......... .......... .......... .......... ..........
43UC02.SEQ  .......... .......... ......T... .C.......G ......A... ..........
43UC04.SEQ  .......... .......... .......... .......... .......... ..........
43UC05.SEQ  .......... .......... .......... .......... .......... ..........
43UC06.SEQ  .......... .......... .......... .......... .......... ..........
43UC07.SEQ  .......... .......... .......... .......... .......... ..........
43UC11.SEQ  .......... .......... ......T... .C.......G ......A... ..........
43UC15.SEQ  .......... .......... .......... .......... .......... ..........
43TA.SEQ    ...CC....G AACAGGC... .......... .......... .G....A..G ..T....T..
```

```
            [2222222222 2222222222 2222222222 2222222222 2222222222 2222222223
             4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    TTAAGTTACC CATGATTGTG GTCTTGTCAT GATCAGTAGT CCAAAAACCA AATCAGGAC-
43U02.SEQ    .......G.. .G........ ..T....... ......C... .......... .....T.C.C
43U03.SEQ    .......G.. .G........ .......... .......... ..-------- .....T.C..
43U04.SEQ    .......G.. .G........ .......... .......... ....G..... .....T.C..
43U05.SEQ    .......G.. .G........ ..T....... ......C... .......... .....T.C.C
43U07.SEQ    .......G.. .G........ .......... .......... ..-------- .....T.C..
43U08.SEQ    .......G.. .G........ .......... .......... ..-------- .....T.C..
43U09.SEQ    .......G.. .G........ .......... .......... ....G..... .....T.C..
43U10.SEQ    .......G.. .G........ ..T....... ......C... .......... .....T.C.C
43U14.SEQ    .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC01.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC02.SEQ   .......G.. .G........ ..T....... ......C... .......... .....T.C.C
43UC04.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC05.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC06.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC07.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43UC11.SEQ   .......G.. .G........ ..T....... ......C... .......... .....T.C.C
43UC15.SEQ   .......G.. .G........ .......... .......... ....G..... .....T.C..
43TA.SEQ     .....G.T.. .G........ ..T....... .......... ....G..... .....T.C.C


            [3333333333 3333333333 3333333333 3333333333 3333333333 3333333333
             0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    AGGTTCGTCG TCACGTCGGT GACTTAGATT ATCCACGGTC TTGGAACTGT CTCCTCTCCT
43U02.SEQ    .......... ....AC.... .......... ..T..T.A.. .......... ..........
43U03.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .A.....T..
43U04.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43U05.SEQ    .......... ....AC.... .......... ..T..T.A.. .......... ..........
43U07.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .A.....T..
43U08.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .A.....T..
43U09.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43U10.SEQ    .......... ....AC.... .......... ..T..T.A.. .......... ..........
43U14.SEQ    ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC01.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC02.SEQ   .......... ....AC.... .......... ..T..T.A.. .......... ..........
43UC04.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC05.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC06.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC07.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43UC11.SEQ   .......... ....AC.... .......... ..T..T.A.. .......... ..........
43UC15.SEQ   ....---.T. ....A....C .......... ..T..T.A.. .......... .......T..
43TA.SEQ     .......... ....A..... .......... ..T..T.A.. .......... ..........
```

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            [3333333333 3333333333 3333333333 3333333334 4444444444 4444444444
             6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    CCCCCATTAA GAAATGCAGA TTCTCACCAG ATCACACGGT ACGTTGTTAG ---CTACCTA
43U02.SEQ    .......... .......... .......... .......... .......... ..........
43U03.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43U04.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43U05.SEQ    .......... .......... .......... .......... .......... ..........
43U07.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43U08.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43U09.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43U10.SEQ    .......... .......... .......... .......... .......... ..........
43U14.SEQ    T...A..... .......... .....G..G. .......... .........C TAA.......
43UC01.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43UC02.SEQ   .......... .......... .......... .......... .......... ..........
43UC04.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43UC05.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43UC06.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43UC07.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43UC11.SEQ   .......... .......... .......... .......... .......... ..........
43UC15.SEQ   T...A..... .......... .....G..G. .......... .........C TAA.......
43TA.SEQ     ....A..... .......... .......... .......... .......... ..........


            [4444444444 4444444444 4444444444 4444444444 4444444444 4444444444
             2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    GCCATATTTT ATGCCTAAAT TATTTTGGGA CATAAAGTGT TGCAGACATC TTTTCGGCGA
43U02.SEQ    .G..C..... .......... .......... .......C.C .......... ..........
43U03.SEQ    ......G.G. .......... .......... .......C.C .......... ..........
43U04.SEQ    ......G.G. .......... .......... .......C.C .......... ..........
43U05.SEQ    .G..C..... .......... .......... .......C.C .......... ..........
43U07.SEQ    ......G.G. .......... .......... .......C.C .......... ..........
43U08.SEQ    ......G.G. .......... .......... .......C.C .......... ..........
43U09.SEQ    .G........ .......... .......... .......C.C .......... ..........
43U10.SEQ    .G..C..... .......... .......... .......C.C .......... ..........
43U14.SEQ    ......G.G. .......... .......... .......C.C .......... ..........
43UC01.SEQ   ......G.G. .......... .......... .......C.C .......... ..........
43UC02.SEQ   .G..C..... .......... .......... .......C.C .......... ..........
43UC04.SEQ   .G........ .......... .......... .......C.C .......... ..........
43UC05.SEQ   .G........ .......... .......... .......C.C .......... ..........
43UC06.SEQ   .G........ .......... .......... .......C.C .......... ..........
43UC07.SEQ   .G........ .......... .......... .......C.C .......... ..........
43UC11.SEQ   .G..C..... .......... .......... .......C.C .......... ..........
43UC15.SEQ   .G........ .......... .......... .......C.C .......... ..........
43TA.SEQ     .......--- -----..... ..A...A... .G.....C.. .......... .....A....
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            [4444444444 4444444445 5555555555 5555555555 5555555555 5555555555
             8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    CATGCCAATG GATGTATCCG AAGAACCTTC AGCTACGAGG AAGAAACAGC ATGCAAAACA
43U02.SEQ    .......... ......G... .......... .......... .......... ..........
43U03.SEQ    .......... ......G... .......... .......... .......... ..........
43U04.SEQ    .......... ......G... .......... .......... .......... ..........
43U05.SEQ    .......... ......G... .......... .......... .......... ..........
43U07.SEQ    .......... ......G... .......... .......... .......... ..........
43U08.SEQ    .......... ......G... .......... .......... .......... ..........
43U09.SEQ    .......... ......G... .......... .......... .......... ..........
43U10.SEQ    .......... ......G... .......... .......... .......... ..........
43U14.SEQ    .......... ......G... .......... .......... .......... ..........
43UC01.SEQ   .......... ......G... .......... .......... .......... ..........
43UC02.SEQ   .......... ......G... .......... .......... .......... ..........
43UC04.SEQ   .......... ......G... .......... .......... .......... ..........
43UC05.SEQ   .......... ......G... .......... .......... .......... ..........
43UC06.SEQ   .......... ......G... .......... .......... .......... ..........
43UC07.SEQ   .......... ......G... .......... .......... .......... ..........
43UC11.SEQ   .......... ......G... .......... .......... .......... ..........
43UC15.SEQ   .......... ......G... .......... .......... .......... ..........
43TA.SEQ     .......... ......G... .......... .......... .......... ..........


            [5555555555 5555555555 5555555555 5555555555 5555555555 5555555556
             4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    ACAGCA---C CGATCGATCT TTAAGGTT-- ------AGAT GTCCAGACCC ACTCCCCAGT
43U02.SEQ    ......GCA. .A........ ........CA CAGCTG..C. .......... ........T.
43U03.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U04.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U05.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U07.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U08.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U09.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U10.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43U14.SEQ    .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC01.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC02.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC04.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC05.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC06.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC07.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC11.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43UC15.SEQ   .......... .A........ ........CA CAGCTG..C. .......... ........T.
43TA.SEQ     .......... .......... ........CA GAGCTG..C. .......... ........T.
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            [6666666666 6666666666 6666666666 6666666666 6666666666 6666666666
             0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    ACAG----GT AAGCAAATGA GCATGGCGAG ATTTGTGCGA CGAGATTAGC TTCCAAGTTG
43U02.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U03.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U04.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U05.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U07.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U08.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U09.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U10.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43U14.SEQ    ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC01.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC02.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC04.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC05.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC06.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC07.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC11.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43UC15.SEQ   ....ACAG.. .......... .......... ........C. GT.....G.T .....G....
43TA.SEQ     ........A. .......... ....C..... .....A..C. .......G.T .....G....


            [6666666666 6666666666 6666666666 6666666667 7777777777 7777777777
             6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    GGAGGTTGAT GGCCACTAAT TAGCCGGTTA GCAATCAGCC AAGTCCACAA GGGCTAGCTA
43U02.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U03.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U04.SEQ    .......... .......... .......... ........-- ------.... .........C
43U05.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U07.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U08.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U09.SEQ    .......... .......... .......... ........-- ------.... ....C..T..
43U10.SEQ    .......... .......... .G........ ........-- ------.... ....C..T..
43U14.SEQ    .......... .......... .......... ........-- ------.... .........C
43UC01.SEQ   .......... .......... .......... ........-- ------.... .........C
43UC02.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC04.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC05.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC06.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC07.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC11.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43UC15.SEQ   .......... .......... .......... ........-- ------.... ....C..T..
43TA.SEQ     .......... .....T.... ........A. .A......-- ------.... ....C..T..
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            [7777777777 7777777777 7777777777 7777777777 7777777777 7777777777
             2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
             1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
43U01.SEQ    ACGAGTACAA CTAACTAGGG -ACAAATCAA TTGAAGGGTT GACAGAAGTT CGGAGTGCT-
43U02.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U03.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U04.SEQ    ..---..... .......... G......... .......... .......... .AT......T
43U05.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U07.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U08.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U09.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U10.SEQ    ..---..... .......... G......... .......... .......... .AT......G
43U14.SEQ    ..---..... .......... G......... .......... .......... .AT......T
43UC01.SEQ   ..---..... .......... G......... .......... .......... .AT......T
43UC02.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC04.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC05.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC06.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC07.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC11.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43UC15.SEQ   ..---..... .......... G......... .......... .......... .AT......G
43TA.SEQ     ..---..... .......... G......... .......... .......... .A.......G


            [7777777777 7777777778 88
             8888888889 9999999990 00
             1234567890 1234567890 12]
43U01.SEQ    CTAACTAATT AAGAGGAACA CC
43U02.SEQ    .......... .......... ..
43U03.SEQ    .......... .......... ..
43U04.SEQ    .......... .......... ..
43U05.SEQ    .......... .......... ..
43U07.SEQ    .......... .......... ..
43U08.SEQ    .......... .......... ..
43U09.SEQ    .......... .......... ..
43U10.SEQ    .......... .......... ..
43U14.SEQ    .......... .......... ..
43UC01.SEQ   .......... .......... ..
43UC02.SEQ   ...??????? ?????????? ??
43UC04.SEQ   .......... .......... ..
43UC05.SEQ   .......... .......... ..
43UC06.SEQ   .......... .......... ..
43UC07.SEQ   .......... .......... ..
43UC11.SEQ   .......... .......... ..
43UC15.SEQ   .......... .......... ..
43TA.SEQ     ..???????? ?????????? ??


;
ENDBLOCK;
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D21 Locus

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#nexus
[Subset data]

BEGIN DATA;
  DIMENSIONS NTAX=17  NCHAR= 1082;
   FORMAT
    INTERLEAVE
      MISSING=? GAP=- MATCHCHAR=.
       DATATYPE=DNA
;
MATRIX
      [
                1 1111111112 2222222223 3333333334 4444444445 5555555556
      1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA    AAAGGACAAT GATCCCGGTA ACAAAGACAA TAACACATGC AATCAATGCA TACAGAATTC
C04   .......... A......... .......... .......... .......... ..........
C10   .......... .......... .......... .......... .......... ..........
C13   .......... A......... .......... .......... .......... ..........
C17   ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C27   ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
UC02  .......... .......... .......... .......... .......... ..........
UC04  .......... A......... .......... .......... .......... ..........
UC05  .......... .......... .......... .......... .......... ..........
UC07  ?????????? .......... .......... .......... .......... ..........
UC08  .......... .......... .......... .......... .......... ..........
UC10  ?????????? ?????????? ????????.. .......... .......... ..........
UC11  ........G. .......... .......... .......... .......... ..........
UC12  ?????????? ???....... .......... .......... .......... ..........
UC13  ?????..... .......... .......... .......... .......... ..........
UC14  ........G. .......... ........T. .......... .......... ..........
UC15  .......... .......... .......... .......... .......... ..........

     [
                                                          1 1111111111 1111111111
      6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
      1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA    CACGCTTCAT ACCATGTTTC CTCCCACCTG ATCCTTCAGG ACTATTTGGT GACGCAGAGC
C04   .......... .......... .......... .......... .......... .....G....
C10   .......... .......... .A........ .......... .......... ..........
C13   .......... .......... .......... .......... .......... .....G....
C17   ?????????? ?????????? ?????????? ?????????? .......... ..........
C27   ?????????? ?????????? ?????????? ?????????? .......... ..........
UC02  .......... .......... .......... .......... .......... ..........
UC04  .......... .......... .......... .......... .......... .....G....
UC05  .......... .......... .......... .......... .......... ..........
UC07  .......... .......... .......... .......... .........G ..........
UC08  .......... .......... .......... .......... .......... ..........
UC10  .......... .......... .......... .......... .........G .....G....
UC11  .......... .......... .......... .......... .......... ..........
UC12  .......... .......... .......... .......... .........G .....G....
UC13  .......... .......... .......... .......... .......... ..........
UC14  .......... .......... .......... .......... .......... .....G....
UC15  .......... .......... .......... .......... .......... ..........
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      [
       1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
       2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GGGGAAGAAC AAGTAGTTCA TTCCCTGGAT GGAAAGATGA ATCAGGAAAT TTCAGCAAAC
C04    .......... .......... .......... .........G .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... ...T...... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
UC08   .......... .......... .......... .......... .......... ..........
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UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [
       1111111111 1111111112 2222222222 2222222222 2222222222 2222222222
       8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     TGCTTGGAAC GGGGCCAGAA AAATTGTTGT ATGATACATT GAACTCTACA AGATCGTCTG
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... ...T...... ..........
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      [
       2222222222 2222222222 2222222222 2222222222 2222222222 2222222223
       4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GGAGGCCATC AGGTATGCTA CCATCAAAAT GGTTGCTTGA CAAGTCAATG TGCAGCAAAT
C04    .......... .......... .......G.. .......... .......... ..........
C10    .......... .......... .....G.... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [
       3333333333 3333333333 3333333333 3333333333 3333333333 3333333333
       0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GCTTTAGCTT GGTGATTGAT GTTGGGATTT GCCCAGTAAA GTTGTTCAGG CAGAGATCCA
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........
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      [
       3333333333 3333333333 3333333333 3333333334 4444444444 4444444444
       6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     ACAACACCAA TCCACTCAAA CGACCGATGC CCGAAGGCAG TGGACCGCCA AAAGAATTGT
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [
       4444444444 4444444444 4444444444 4444444444 4444444444 4444444444
       2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     TTGAAAGATC AAGAAATGAA AGGTTTGAAG TCTGTACAGG AAGAACTGAC AAGTCTATAG
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    ........C. .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ...C......
UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......T.. .......... .......... ..........
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      [
       4444444444 4444444445 5555555555 5555555555 5555555555 5555555555
       8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     TAGTTGAATT GTTGGTTTCA GAACTGGGAA GAGGCAGGGT TCCTGCGAAA TTGTTTCCTG
C04    .......... .C........ .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
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UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [
       5555555555 5555555555 5555555555 5555555555 5555555555 5555555556
       4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     AAAGATTAAG ATAGGTCAGC TTAGCTGCTG TAAAGAGATT TTTCGGTAAA GCTCCATGCA
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... ........C. .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
UC04   .......... .......... .......... .......... .......... ..........
UC05   .......... .......... .......... .......... .......... ..........
UC07   .......... .......... .......... .......... .......... ..........
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UC10   .......... .......... .......... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .......... ...GA..... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........
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      [
       6666666666 6666666666 6666666666 6666666666 6666666666 6666666666
       0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GCTGATTGAG ACTAAGATCT ATGGCAATCA ACTCGGGATA GCTTCCGAGA ACAGTC-GGC
C04    .......... .......... .......... .......... .........G ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
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UC12   .......... .......... .......... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ......C...


      [
       6666666666 6666666666 6666666666 6666666667 7777777777 7777777777
       6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     AATTCTCCTG CTAGCAAGTT ATCAGAAATC CTCAATGGAA GGTCAGCCTC AAAAA-TTGG
C04    .......G.. .......... .......... .......--. A......... ..........
C10    .......G.. .......... .......... .......--. A......... ..........
C13    .......... .......... .......... .......--. A......... ..........
C17    .......... .......... ..T....... .......--. A......... ..........
C27    .......... .......... .......... .......--. A......... ..........
UC02   .......... .......... .......... .......--. A......... ..........
UC04   .......... .......... .......... .......--. A......... ..........
UC05   .......... .......... .......... .......--. A......... ..........
UC07   .......... .......... .......... .......--. A......... .....A....
UC08   .......... .......... .......... .......--. A......... ..........
UC10   .......... .......... .......... .......--. A......... ..........
UC11   .......... .......... .......... .......--. A......... ..........
UC12   .......... .......... .......... .......--. A......... ..........
UC13   .......... .......... .......... .......--. A......... ..........
UC14   .......... .......... .......... .......--. A......... ..........
UC15   .......... .......... .......... .......--. A......... ..........
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      [
       7777777777 7777777777 7777777777 7777777777 7777777777 7777777777
       2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GTAGTCTGAT CGGGGCCAGG TTCCTGTTAA CCTATTTGAG CTCAAATCAA TCATCTCAAC
C04    .......... .-........ .......... .......... .......... ..........
C10    .......... .-........ .......... .......... .......... ..........
C13    .......... .-........ .......... .......... .......... ..........
C17    .......... .-........ .......... .......... .......... ..........
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UC11   .......... .-........ .......... .......... .......... ..........
UC12   .......... .-........ .......... .......... .......... ..........
UC13   .......... .-........ .......... .......... .......... ..........
UC14   .......... .-........ .......... .......... .......... ..........
UC15   .......... .-........ .......... .......... .......... ..........


      [
       7777777777 7777777778 8888888888 8888888888 8888888888 8888888888
       8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     ATAATCTCCC CATGTATGGA CAACTGATAT GTTCCCTGAA AGCATGTTTC TACTCAAATC
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .........C. .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
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UC14   .......... .......... .......... .......... .......... ..........
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      [
       8888888888 8888888888 8888888888 8888888888 8888888888 8888888889
       4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     AACCACTGAA CAGCTCCCAA ATGTGATTGG TAAAGTTCCC TGAAGGCTGT TACATGACAG
C04    .......... .......... .......... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..........
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
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UC14   .......... .......... .......... .......... .......... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [
       9999999999 9999999999 9999999999 9999999999 9999999999 9999999999
       0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     ATTCAGGTAC TTCAGATTCA CTGATGTAAC ACGCCTAATT GGACCTGTGT AATACAAGGG
C04    .......... .......... .......... .......... .......... ..........
C10    ..A....... .......... .......... .......... .......... .......C..
C13    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .......... .......... .......... ..........
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UC04   .......... .......... .......... .......... .......... ..........
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UC14   .......... .......... .......... .......... .......... ..........
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      [                                                  1 1111111111 1111111111
       9999999999 9999999999 9999999999 9999999990 0000000000 0000000000
       6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     TTTAGATAAA ATTAGAGGCA ATCGACATCA TGATTAGTAA TCGCAACACA TCATCATTTA
C04    .......... .......... .....G.... .......... .......... ..........
C10    .......... .......... .......... .......... .......... ..T.......
C13    .......... .......... .....G.... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
C27    .......... .......... .....G.... .......... .......... ..........
UC02   .......... .......... .......... .......... .......... ..........
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UC08   .......... .......... .......... .......... .......... ..........
UC10   .......... .......... .....G.... .......... .......... ..........
UC11   .......... .......... .......... .......... .......... ..........
UC12   .......... .......... .....G.... .......... .......... ..........
UC13   .......... .......... .......... .......... .......... ..........
UC14   .......... .......... .....G.... .......... ......T... ..........
UC15   .......... .......... .......... .......... .......... ..........


      [1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 11
       0000000000 0000000000 0000000000 0000000000 0000000000 0000000000 00
       2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 00
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 12]
TA     CAAACATAGT ATGGATTGAT TCAGTGTTAT AGAGGTTATT ATGGCAACAA CAAAAAAACG AG
C04    .......... .......... .......... .......... .......... .......... ..
C10    .......... .......... .......... .......... .......... .......... ..
C13    .......... .......... .......... .......... .......... .......... ..
C17    .......... .......... .......... .......... .......... .......... ..
C27    .......... .......... .......... .......... .......... .......... ..
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UC04   .......... .......... .......... .......... .......... .......... ..
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UC08   .......... .......... .......... .......... .......... .......... ..
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UC12   .......... .......... .......... .......... .......... .......... ..
UC13   .......... .......... .......... .......... .......... .......... ..
UC14   .......... .......... .......... .......... .......... .......... ..
UC15   .......... .......... .......... .......... .......... .......... ..


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ENDBLOCK;
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U6/R6 Locus

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#nexus
[Subset data]

BEGIN DATA;
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   FORMAT
    INTERLEAVE
      MISSING=? GAP=- MATCHCHAR=.
       DATATYPE=DNA
;
MATRIX

        [
                1 1111111112 2222222223 3333333334 4444444445 5555555556
        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA      GCTGCCGAAT TGGCCTATTT CTTGCGCGTA CCAATTGAAG TATTTTGAGG TATCTTTTTT
C01     ?????????? ?????????? .......... .......... .......... ..........
C02     .......... .......... .......... .......... .......... ..........
C03     .......... .......... .......... .......... .......... ..........
C08     ?????????? ?????????? ?????????? ?????????? ?????????? -.........
C17     ?????????? ????????.. .......... .......... .......... ..........
U01     .......... .......... .......... .......... .......... ..........
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U07     ?????????? ?????????. .......... .......... .......... ..........
U08     ?????????? ?????????? ?????????? ?????????? ?????????? ?????????.
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U17     .......... .......... .......... .......... .......... ..........
UC01    .......... .......... .......... .......... .......... ..........
UC02    .......... .......... .......... .......... .......... ..........
UC03    ?????????? ?????????? ?......... .......... .......... ..........
UC04    .......... .......... .C........ .......... ........A. ..........
UC05    .......... .......... .......... .......... .......... ..........
UC06    .......... .......... .......... .......... .......... ..........
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UC08    ?????????? ?????????? .......... .......... .......... ..........
UC11    ?????????? ?......... .......... .......... .......... ..........
UC15    .......... .......... .......... .......... .......... ..........
wh      .......... .......... .......... ......C... .......... ..........
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      [                                                  1 1111111111 1111111111
       6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     GAACTGAGTT GAATGAAGAA AA-GAAGAAT TGGAAGAAGA AAAGTTTTCT CAACACGGGG
C01    .......... .......... .......... .......... ...A...... ..........
C02    .......... .........-- --.------- ---------- ---------- ----------
C03    .......... .........-- --.------- ---------- ---------- ----------
C08    .......... .......... .......... .......... ...A...... ..........
C17    .......... .......... .......... .......... .......... ..........
U01    .......... .......... .......... .......... ...A...... ..........
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U04    .......... .......... .......... .......... ...A...... ..........
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U09    .......... .......... .......... .......... ...A...... ..........
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U12    .......... .......... .......... .......... ...A...... ..........
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U17    .......... .........-- --.------- ---------- ---------- ----------
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UC02   .......... .......... .......... .......... ...A...... ..........
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UC04   .......... .........-- --.------- ---------- ---------- ----------
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UC06   .......... .........-- --.------- ---------- ---------- ----------
UC07   .......... .........-- --.------- ---------- ---------- ----------
UC08   .......... .......... .......... .......... ...A...... ..........
UC11   .......... .......... .......... .......... ...A...... ..........
UC15   .......... .........-- --.------- ---------- ---------- ----------
wh     .......... .......... ..A....... .......... .......... ..........


      [1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
       2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA     AGGAAGTCCC TTCTAAATTG GATTTGTTAT TGTAAGGGGA TTTTTAAGTA TTTATCTAAA
C01    .......... .......... .......... .......... .......... ..........
C02    ---------- ---------- ---------- ---------- ---------- ----------
C03    ---------- ---------- ---------- ---------- ---------- ----------
C08    .......... .......... .......... .......... .......... ..........
C17    .......... .......... .......... .......... .......... ..........
U01    .......... .......... .......... ......T... .......... ..........
U02    .......... .......... .......... ......T... ...C...... ..........
U03    .......... .......... .......... ......T... .......... ..........
U04    .......... .......... .......... ......T... .......... ..........
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UC15   ---------- ---------- ---------- ---------- ---------- ----------
wh     .......-.. ....-..... .......... .......... -......... -.........
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        [1111111111 1111111112 2222222222 2222222222 2222222222 2222222222
         8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA       GGAAGGAACA AACGAGGATA AGAGAAATTT GCTTTTAATT TTTTTTTATC CAAGTGAGAT
C01      .......... .......... .......... .......... .......... ..........
C02      ---------- ---------- ---------- ---------- ---------- ----------
C03      ---------- ---------- ---------- ---------- ---------- ----------
C08      .......... .......... .......... .......... .......... ..........
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UC15     ---------- ---------- ---------- ---------- ---------- ----------
wh       .......... .......... .......... ..-....... .......... ..........


        [2222222222 2222222222 2222222222 2222222222 2222222222 2222222223
         4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA       ATATCGCATA CTATTCTTCC TTTTTCATCC GAAAGGGCTT TTTTTTT-AT TCTATTTCAC
C01      .......... .......... .......... .......... .......T.. ..........
C02      ---------- ---------- ---------- ---------- -------.-- ----------
C03      ---------- ---------- ---------- ---------- -------.-- ----------
C08      .......... .......... .......... .......... .......T.. ..........
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U01      .......... .......... .......... .......... .......... ..........
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UC11     .......... .......... .......... .......... .......... ..........
UC15     ---------- ---------- ---------- ---------- -------.-- ----------
wh       .......... .--A...... -......... ....-..... .......... ..........
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        [3333333333 3333333333 3333333333 3333333333 3333333333 3333333333
         0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA       TATTTCATTC CATCTAGATC TAAGAAAGAA CCCAATGCAC TGAAATTCCA CTACTAACTA
C01      .......... .......... .......... .......... .......... .A.A......
C02      ---------- ---------- ---------- ---------- ---------- ----------
C03      ---------- ---------- ---------- ---------- ---------- ----------
C08      .......... .......... .......... .......... .......... .A.A......
C17      .......... .......... .......... .......... .......... ..........
U01      .......... .......... .......... .......... .......... .A.A......
U02      .......... .......... .......... .......... .......... .G.A......
U03      .......... .......... .......... .......... .......... .A.A......
U04      .......... .......... .......... .......... .......... .A.A......
U05      .......... .......... .......... .......... .......... .A.A......
U07      .......... .......... .......... .......... .......... .A.A......
U08      .......... .......... .......... .......... .......... .A.A......
U09      .......... .......... .......... .......... .......... .A.A......
U10      .......... .......... .......... .......... .......... .A.A......
U12      .......... .......... .......... .......... .......... .A.A......
U14      .......... .......... .......... .......... .......... .A.A......
U17      ---------- ---------- ---------- ---------- ---------- ----------
UC01     ---------- ---------- ---------- ---------- ---------- ----------
UC02     .......... .......... .......... .......... .......... .A.A......
UC03     .......... .......... .......... .......... .......... ...A......
UC04     ---------- ---------- ---------- ---------- ---------- ----------
UC05     .......... .......... .......... .......... .......... .A.A......
UC06     ---------- ---------- ---------- ---------- ---------- ----------
UC07     ---------- ---------- ---------- ---------- ---------- ----------
UC08     .......... .......... .......... .......... .......... .A.A......
UC11     .......... .......... .......... .......... .......... .A.A......
UC15     ---------- ---------- ---------- ---------- ---------- ----------
wh       .......... .......... .......... .......... .......... ...A......


        [3333333333 3333333333 3333333333 3333333334 4444444444 4444444444
         6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA       ATATACAAAA AAGAAGAATA GATACAGGGT ATCAAACCTA TATAGTTTTT GCTTCAAAGA
C01      ..???????? ?????????? ?????????? ?????????? ?????????? ??????????
C02      --------.. .......... .......... .......... ..G....... ..........
C03      --------.. .......... .......... .......... ..G....... ..........
C08      ..???????? ?????????? ?????????? ?????????? ?????????? ??????????
C17      .......... .......... .......... .......... .......... ..........
U01      .......... .......... .......... .......... ..G....... ..........
U02      .......... .......... .......... .......... ..G....... ..........
U03      .......... .......... .......... .......... ..G....... ..........
U04      .......... .......... .......... .......... ..G....... ..........
U05      .......... .......... .......... .......... ..G....... ..........
U07      .......... ..........  .????????? ?????????? ?????????? ??????????
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U09      .......... .......... .......... .......... ..G....... ..........
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U12      .......... .......... .......... .......... ..G....... ..........
U14      .......... .......... .......... .......... ..G....... ..........
U17      --------.. .....T.... .......... .......... ..G....... ..........
UC01     --------.. .......... .......... .......... ..G....... ..........
UC02     .......... .......... .......... .......... ..G....... ..........
UC03     .......... .......... .......... .......... ..G....... ..........
UC04     --------.. .......... .......... .......... ..G....... ..........
UC05     .......... .......... .......... .......... ..G....... ..........
UC06     --------.. .......... .......... .......... ..G....... ..........
UC07     --------.. .......... .......... .......... ..G....... ..........
UC08     .......... .......... ...C...... .......... ..G....... ..........
UC11     .......... .......... .......... .......... ..G....... ..........
UC15     --------.. .......... .......... .......... ..G....... ..........
wh       .......... .......... .......... .......... ..G....... ..........
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        [4444444444 4444444444 4444444444 4444444444 4444444444 4444444444
         2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA       AATAGAAATA TCAT------ ---------- CATATAGAGT CAGGGAATGG AATAGAGTCA
C01      ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C02      .......... ....GAAATA GAAATATCAT .......... .......... ..........
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U01      .......... ....GAAATA GAAATATCAT .......... .......... ..........
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U17      .......... ....GAAATA GAAATATCAT .......... .......... ..........
UC01     .......... ....GAAATA GAAATATCAT .......... .......... ..........
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UC08     .......... ....GAAATA GAAATATCAT ...??????? ?????????? ??????????
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UC15     .......... ....GAAATA GAAATATCAT .......... .......... ..........
wh       .......... ....GAAATA GAAATATCAT .......... .......... ..........


        [4444444444 4444444445 5555555555 5555555555 5555555555 5555555555
         8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
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TA       GCGAATGAAG CATATTCATT AACAACTCCA TTTACGGATC AAAAATGAAA AAAAAGAAAG
C01      ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
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U01      .......... .......... .......... .....A.... .......... ..........
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UC01     .......... .......... .......... .....A.... .......... ..........
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UC05     .......... .......... .......... .....A.... .......... ..........
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UC07     .......... .......... .......... .....A.... .......... ..........
UC08     ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
UC11     .......... .......... .......... .....A.... .......... ..........
UC15     .......... .......... .......... .....A.... .......... ..........
wh       .......... .......... .......... .....A.... .......... ..........
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        [5555555555 5555555555 5555555555 5555555555 5555555555 5555555556
         4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
         1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 ]
TA      CATTGCCTTC TTTACTATAT CTTGTATTTA TCGTACTTTT GCCTTGGGGG GTCTCTTCCT
C01     ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
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UC05    .......... .......... .......... .......... .......... ..........
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        [6666666666 6666666666 6666666666 6666666666 6666666666 6
         0000000001 1111111112 2222222223 3333333334 4444444445 5
         1234567890 1234567890 1234567890 1234567890 1234567890 1]
TA      CATTTAACAA ATGTCTGGAA CTTTGGATTA AGAATTGGTG GAATACCAGG C
C01     ?????????? ?????????? ?????????? ?????????? ?????????? ?
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U09     .......... .......... .......... .......... .......... .
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ENDBLOCK;
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